Spring 2020

Influence of Stormwater Management on Oxygen Demand and Organic Matter Cycling in Urbanizing Headwater Catchments

Kelly McCabe

INFLUENCE OF STORMWATER MANAGEMENT ON OXYGEN DEMAND AND ORGANIC MATTER CYCLING IN URBANIZING HEADWATER CATCHMENTS

by

Kelly McCabe

Bachelor of Science
University of South Carolina, 2016

Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in

Marine Science

College of Arts and Sciences

University of South Carolina

2020

Accepted by:

Claudia Benitez-Nelson, Director of Thesis

Erik Smith, Reader

Susan Lang, Reader

Christopher Osburn, Reader

Cheryl L. Addy, Vice Provost and Dean of the Graduate School

ACKNOWLEDGEMENTS

This thesis was supported by the South Carolina Sea Grant Consortium grant and additional funding from the University of South Carolina (UofSC) School of Earth, Ocean, and Environment and the UofSC Belle W. Baruch Institute for Marine and Coastal Sciences (Franklin H. and Virginia M. Spivey Undergraduate Fellowship and F. John Vernberg Bicentennial Graduate Fellowship). The completion of this work was made possible with the help of many: undergraduate researchers, Nicole Weirich and Brittany Morse; laboratory technicians, Susan Canady-Denham, Julie Krask, and Meryssa Piper; and my co-advisors, Claudia Benitez-Nelson and Erik Smith.

ABSTRACT

Excessive organic matter (OM) is driving declining dissolved oxygen (DO) concentrations in coastal ecosystems, worldwide. The quantity, source, and composition of OM transported to coastal ecosystems via stormwater runoff has been altered by land use changes associated with urbanization and subsequent headwater alterations that accompany urban stormwater management. To understand the role of stormwater runoff in the decline of coastal DO, biochemical oxygen demand (BOD) was determined for samples collected during rain events from the outfalls of a variety of stormwater infrastructure with watersheds spanning a range of development. Measurements of particulate and dissolved carbon and nitrogen and chlorophyll-*a* concentrations as well as stable carbon isotope ($\delta^{13}C$) values helped explain the drivers of stormwater BOD concentrations. Results suggest stormwater runoff is a significant source of labile OM to receiving waters, however BOD concentrations vary greatly both among and within sites in response to rain events. This variability in BOD was best predicted by concentrations of particulate OM (POM), especially chlorophyll-*a* concentrations, rather than the larger dissolved fraction of OM. These findings stress the importance of managing episodic stormwater pollutant discharge, especially POM, from urbanized areas to mitigate DO impairment in larger downstream systems.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Urbanization is a leading cause of water quality degradation [*Walsh et al.*, 2005] in coastal riverine and marine ecosystems, especially with respect to low dissolved oxygen (DO). The level of DO in coastal waters is controlled by a variety of natural processes, as well as by urban anthropogenic point and nonpoint source discharge of allochthonous organic matter (OM) and autochthonous OM production enhanced by nutrient pollution. The quantity and especially the composition of the supplied OM determines the amount and rate of oxygen removal by microbial aerobic respiration [*del Giorgio and Davis*, 2003]. OM is comprised of a diverse array of compounds divided into particulate organic matter (POM) and dissolved organic matter (DOM) size fractions with compounds ranging from simple bioavailable forms (e.g., sugars, proteins, and amino acids) that are readily catabolized relative to more complex forms (e.g., humic substances) [*del Giorgio and Cole*, 1998; *Benner*, 2003, 2007; *del Giorgio and Davis*, 2003]. When the respiration of the various supplied OM compounds is sufficient enough to drive daily average DO concentrations below 5 mg L^{-1} ($\leq$ 4 mg L^{-1} for blackwater systems), the threshold often used to define DO impairment [*U.S. EPA*, 1995; *SCDHEC*, 2014], aquatic life becomes stressed. Once DO concentrations reach hypoxic levels (< 2 mg L^{-1}) major mortality events are triggered [*Diaz and Rosenberg*, 1995; *Breitburg et al.*, 2018].

Consistent with increased urbanization throughout the 20th and 21st century, coastal water DO levels have declined, leading to an increase in the number, size, duration, and severity of hypoxic zones globally [*Diaz and Rosenberg*, 2008; *Doney*, 2010; *Breitburg et al.*, 2018]. In the United States, the rise in impaired and hypoxic waters has led to increasingly stringent regulations on pollution from altered landscapes as outlined in the Congressional Clean Water Act of 1972 [*U.S. Congress*, 2002] and administered by the U.S. Environmental Protection Agency (USEPA). The USEPA administers the National Pollution Discharge Elimination System (NPDES) permitting program and Total Maximum Daily Load (TMDL) plans, which set limits on pollutants discharged from a given source or that can enter a receiving water body and still maintain sufficient DO levels for sustaining aquatic life [*US EPA*, 1995, 2016]. These regulations rely on biochemical oxygen demand (BOD), the conventional measure of OM decomposition by heterotrophic microbes in polluted waters, to establish effective limits. BOD is defined as the concentration of oxygen consumed by microbial decomposition of OM and the conversion of nitrogen compounds to the stable forms of nitrite and nitrate over a specified incubation period at 20°C [*Delzer and Mckenzie*, 2003]. Thus, BOD defines the amount of biodegradable OM in a water sample. While much is known about point source pollution BOD concentrations, less is known about nonpoint source BOD concentrations, which hinders the effectiveness of NPDES permits and TMDL plans.

Runoff water during storm events is a primary transport mechanism delivering nonpoint source OM from land to receiving waters. The replacement of once permeable natural landscapes with impervious surfaces such as roads, parking lots, and buildings alter natural hydrologic regimes by increasing the volume and velocity of stormwater flow

across land surfaces [*Leopold*, 1968; *Schueler*, 1994; *Walsh et al.*, 2005]. This mobilizes large quantities of OM from land into aquatic systems, especially during the initial pulse of runoff and during intense storms [*Walsh et al.*, 2005; *Kaushal et al.*, 2014; *Smith and Kaushal*, 2015]. In order to reduce the transport of OM via urban stormwater runoff and comply with water quality regulations, best management practices (BMPs; e.g. retention ponds and constructed wetlands) have been integrated into the complex networks of more traditional engineered stormwater conveyances (e.g., pipes, culverts, and ditches) during new development. BMPs are designed to reduce peak water discharge or the first flush. Improved water quality is assumed to accompany the reduction of first flush effects by resulting in a decrease in the amount of OM carried in runoff that then drives stormwater BOD concentrations [*Smith et al.*, 2013]. Research suggests implementing BMPs may not be enough to reduce the overall volume of stormwater runoff and subsequent release of OM from land to downstream coastal systems due to the coverage of impervious surfaces, density and connectivity of modern stormwater flow paths, and climatic change [*McCuen*, 1979; *Emerson et al.*, 2005; *O'Geen et al.*, 2010; *Smith et al.*, 2013; *Meierdiercks et al.*, 2017]. Indeed, the source, magnitude, and biodegradability of OM discharge from various stormwater catchments remains relatively ambiguous.

Dissimilar landscapes produce different organic compounds that could be transported to aquatic systems and drive low DO [*Williams et al.*, 2010; *Petrone et al.*, 2011; *McElmurry et al.*, 2014; *Bhattacharya and Osburn*, 2020]. A growing body of literature suggests urban landscapes may be enhancing the bioavailability of OM in aquatic systems (e.g., *Wiegner and Seitzinger*, 2004; *Hudson et al.*, 2008; *Hosen et al.*, 2014; *McElmurry et al.*, 2014; *Khamis et al.*, 2017]), but understanding the changes in OM source

and lability associated with increasing urbanization and the subsequent effect on aquatic oxygen demand, as defined by BOD, remains uncertain. Some of this ambiguity stems from the complex transport pathways within the coastal environment that include the networks of modern stormwater infrastructure that may transform, trap, or even contribute to the DOM exports from watersheds [*McElmurry et al.*, 2014]. Some research has shown that stormwater BMPs effectively retain suspended sediment and nutrient inputs from land [*Meierdiercks et al.*, 2017; *Schroer et al.*, 2018], but transformations within BMPs including POM leaching to DOM [*Tranvik et al.*, 2009; *Downing*, 2010; *Lusk and Toor*, 2016], microbial and photochemical degradation [*Obernosterer and Benner*, 2004; *Smith and Benner*, 2005; *Díaz et al.*, 2008; *Downing*, 2010], and autochthonous production [*Mallin et al.*, 2002; *Lewitus et al.*, 2008] can make BMPs significant sources of labile OM to receiving waters. Thus, effective stormwater and coastal management requires an understanding of how both land cover alterations and the type of stormwater control measure influence the OM composition and BOD concentrations of nonpoint source discharge.

The goal of this study is to quantify stormwater runoff BOD concentrations and identify the OM sources driving oxygen utilization. This knowledge is critical for understanding the contribution of stormwater runoff to coastal DO impairment and supporting effective management of coastal waters. In this study, the quantity, composition, and lability of OM in stormwater runoff are examined relative to differing stormwater control structures associated with increasing urbanization in the coastal plain of South Carolina (SC). The SC coast, like many coastal regions, is experiencing rapid urbanization. Despite the construction of various stormwater infrastructure, including a rise

in BMP retention ponds [*Schroer et al.*, 2018], SC now identifies low DO as the primary water quality issue impacting aquatic life within the coastal riverine and marine waters of all eight of the state's coastal counties [*SCDHEC*, 2014, 2018]. In fact, the oxygen concentrations continue to decline in the Waccamaw River (USGS 02110802) a coastal plain blackwater system that flows south from southeast North Carolina to Winyah Bay at Georgetown, SC, despite the establishment of a TMDL plan back in 1999 [*SCDHEC*, 1999] and issuing NPDES permits for nonpoint sources in more recent years [*Horry County*, 2014; *Georgetown County*, 2015]. Our results show high BOD concentrations in stormwater runoff, especially during the first flush of runoff that drains into the Waccamaw River and the SC coastal North Atlantic. BOD concentrations combined with OM concentrations and characterization demonstrate current stormwater infrastructure does not adequately reduce nonpoint source BOD concentrations due to an abundance of labile OM, especially POM, laterally transported from land as well as from OM production within the stormwater infrastructure.

CHAPTER 2

METHODS

2.1 *Study Sites*

During the summer of 2018 (June 1st-August 1st), stormwater runoff was collected along the hydrographs of six rain events from 16 stormwater catchments located in South Carolina's Horry and Georgetown counties (Figure 2.1). In the late spring and early summer of 2019 (May 1st-August 1st), runoff was sampled during another eleven storms at the same 16 stormwater catchments as well as 4 additional sites to capture the diversity of catchment structures and micro-watershed land cover characteristics in this region (Figure 2.1; Table 2.1). Combined, the 20 sites include a range of engineered stormwater conveyances, best management practices, and micro-watersheds spanning undeveloped forests and wetlands to high density urban developments with high percentages of impervious surfaces (Figure 2.1; Table 2.1). All of the stormwater catchments lie within the coastal plain of the lower Pee Dee watershed (Hydrologic unit code 8), and either drain into the North Atlantic via coastal marine embayments between the Murrells Inlet and the North Inlet estuaries or via the Waccamaw River at Winyah Bay (Figure 2.1).

The individual site drainage basins were manually delineated in ArcGIS 10.6.1 according to the eight direction matrix (D8) approach that relies on a continuous drainage network of cells connected to only one neighboring cell [*Tribe*, 1992]. Horry and Georgetown county LiDAR data (3 m × 3 m cell size) collected by the South Carolina Department of Natural Resources (SCDNR) in 2009 and 2017, respectively, comprised

the digital elevation model (DEM) matrix for our watershed delineation approach (www.dnr.sc.gov/GIS/lidarstatus.html; Figure 2.1). Additional information from local stormwater managers and field observations (e.g. community and development watershed plans, flow direction, etc.) confirmed the accuracy of these ArcGIS delineations. The 2016 National Land Cover Database (NLCD) that defines percent impervious surface coverage (%IC) on a 30 m x 30 m scale [*Yang et al.*, 2018] was used to assess the effect of drainage basin land use characteristics on nonpoint source pollution at each site. Table 2.1 outlines the characteristics of the individual micro-watersheds including the average %IC (%IC range = 0% to ~43%).

2.2 ***Sample collection and processing***

The sampling season was confined to the late spring and summer (May-August), a period of high precipitation [*Harder et al.*, 2007] and the onset of the seasonal DO decline in the Waccamaw River [*SCDHEC*, 1999]. Stormwater samples from individual catchments were collected along the rising and falling limb of rain event hydrographs as captured by HOBO Water Level Data Loggers (Onset Corp, USA) at the majority of sites. Acid-cleaned 1000 mL self-sealing HDPE Nalgene stormwater sampler bottles (Thermo Scientific, USA) were mounted within a rain proof casing at the outlet of individual stormwater catchments prior to anticipated rain events. As the water level rose within a catchment (termed the rising limb), the bottles sampled the initial pulse or first flush of stormwater runoff. All sample bottles were retrieved within 16 h of the start of the precipitation event. Retrieval times varied based on the timing, duration, and intensity of a given rain event. An additional 1000 mL of manually collected samples were retrieved

after each rain event as the water levels in the catchments began to recede (termed the falling limb).

All samples were stored in an ice filled cooler during the collection process (~3 h). The samples were then equilibrated to 20°C with a water bath (~1 h) for immediate processing. The pH and percent oxygen (O_2) saturation at 20°C were recorded; samples with a percent O_2 saturation below 90% were bubbled with O_2 to saturation (90-100%). Temperature and oxygen equilibrated samples were then transferred to borosilicate glass BOD bottles (300 ml nominal volume) for the determination of both 5 d BOD (BOD5) and ultimate BOD (UBOD). Remaining sample water was filtered through 25 mm diameter, ~0.7 μm particle retention, pre-combusted, glass fiber filters (GF/F) under low vacuum. Operationally, all the compounds larger than 0.7 μm trapped on the filter were defined as the particulate fraction, while all the compounds that passed through the GF/F were defined as the dissolved fraction. Two filters were stored frozen (-80°C) until further analyses of particulate carbon and nitrogen concentrations as well as $\delta^{13}C$ isotopic composition. An additional filter was stored in a 20 mL HDPE scintillation vial in the dark at -20°C for up to 24 h before chlorophyll-*a* analysis. The filtrate was subsampled to quantify dissolved organic carbon (DOC), total dissolved nitrogen (TDN) and inorganic nitrogen (DIN) concentrations as well as to characterize the DOM using DOC stable isotopes ($\delta^{13}C$-DOC). All filtrate subsamples were stored frozen at -80°C until further analysis.

2.3 ***Biochemical Oxygen Demand (BOD) incubations***

The BOD5 values were evaluated according to Standard Method 5210 B [*Eaton et al.*, 2005]. No artificial bacterial seed population additions nor sample dilutions were made in this experiment per Standard Method 5210 C [*Eaton et al.*, 2005]. Nutrient buffer

additions were added in 2018, but not in 2019 (see Appendix A). Samples were kept in 300 mL borosilicate glass bottles with a DO sensor membrane affixed to the inside of the bottle. The sample bottles were placed on shaker tables within thermostatically controlled air incubators kept at 20°C ± 1°C. DO was monitored over 5 d using a Wiltrox chemiluminescent sensor spot system (Loligo Systems, Denmark), which reads the DO concentration externally from the sensor membrane on the inside edge of the bottle. This method eliminates the need to open the bottle for probe based measurements and thus allows reliable repeated DO measurements on the same sample. The chemiluminescent system has a DO percent saturation accuracy of ± 0.4% and precision of ± 0.1%. Since no seed culture was added and no dilutions were made, BOD5 was calculated with Equation 1.

$$BOD_5 = (D_1 - D_2) \tag{1}$$

where D_1 is the initial DO concentration in mg L^{-1}, D_2 is the final DO concentration after 5 d of incubation in mg L^{-1} [*Eaton et al.*, 2005]. BOD5 triplicate values had a mean coefficient of variability (CV) of 5 ± 4%.

To observe the extended degradation of OM and to evaluate BOD degradation kinetics, incubations without nutrient buffer additions were continued for a total of 28 d (see Appendix A). This 28 d incubation is operationally defined as UBOD, which is equivalent to the amount of DO ultimately consumed by respiratory and nitrification processes [*Cox*, 2003]. Following Standard Methods 5210 C [*Eaton et al.*, 2005], now with repeated DO measurements made possible with the Wiltrox system, UBOD kinetics were measured directly. Bottles were only opened for re-aeration, on those occasions when the DO concentration dropped below 1.5 mg L^{-1} during the incubation. Conventional BOD

degradation is estimated by a first-order kinetic decay equation (Equation 2) to define individual sample BOD decay kinetics.

$$BOD_t = UBOD(1 - e^{-kt}) \quad (2)$$

where BOD_t is the measured BOD at time t, UBOD is the ultimate BOD consumed, k is the exponential decay coefficient, and t is the time since the start of the incubation [*Eaton et al.*, 2005]. Triplicate UBOD values had a 4 ± 2% mean CV. These UBOD concentrations were converted to concentrations of total organic carbon (TOC) consumed using the average respiration quotient for freshwater systems described by Berggren et al. [2012], where 1.2 moles of O_2 removed equates to 1 mole of organic C respired (1.2 O_2 : 1 C).

2.4 ***Particulate carbon and nitrogen analyses***

Particulate C and N filters were dried and prepared following the methods of Hedges and Stern [1984] and did not include the filter digestion with 10% hydrochloric acid (HCl) to remove inorganic C. SC stormwater catchments contain a negligible amount of inorganic C, as found by Schroer et al [2018] and further confirmed by testing of paired acidified and un-acidified samples in this study (paired t-test, n = 9; $p \geq 0.05$). Thus, total particulate C was assumed equal to particulate organic C (POC). After the filters were dried at 50°C for at least 24 h, C and N were evaluated simultaneously with an atropine standard curve (Costech #031042; 70.56% C, 4.84% N) on a Costech ECS 4010 Elemental analyzer. The standard reference material, Buffalo River Sediment (NIST RM 8704; 3.35% C), and blank combusted GF/F filters were run in tandem with the samples. Additionally, a subset of replicate sample runs (~10%) had a mean analytical error of 9 ± 8% CV for C and 10 ± 10% CV for N.

After filters were dried at 50°C for at least 24 h, they were also analyzed using an elemental combustion analyzer attached to an isotopic ratio mass spectrometer (IRMS) to determine the isotope composition of $\delta^{13}C$ [*Kendall et al.*, 2001]. $\delta^{13}C$ values were determined with sucrose (-12.4 ‰) and USGS standards (Graphite, -16.05‰; Glutamic acid, -26.39‰) prepared over a concentration range that encompassed the samples. Particulate C isotopic compositions were expressed in per mil notation relative to the international atmospheric air standard for N and the Vienna Pee Dee Belemnite (VPDB) international standard for C [*Kendall et al.*, 2001].

2.5 *Chlorophyll-a determination*

The chlorophyll-*a* measurements were modified from U.S. EPA method 445.0 [*Arar and Collins*, 1997]. In brief, 10 mL of 90% acetone was added to each 20 mL HDPE scintillation vial to cover the GF/F filter. The samples were then stored upside down in a dark cooler at 2°C for 24 h. The samples were shaken periodically throughout the 24 h storage period to facilitate extraction. The chlorophyll-*a* concentrations were analyzed using a Turner Trilogy Laboratory Fluorometer with a chlorophyll acidified/non-acidified application module (model #7200-046). About 7% of the samples were run in duplicate with a mean analytical error of 7 ± 7% CV.

2.6 *Dissolved carbon and nitrogen analyses*

Total dissolved nitrogen (TDN) measurements were run on a Technicon Nutrient Auto Analyzer with a SEAL analytical XY-2 sampler according to Standard Methods SM 4500-N C [*Eaton et al.*, 2005] after an alkaline potassium persulfate oxidation as described by Eaton and others [2005]. Total dissolved N quality control checks and spikes

accompanied each run. Approximately 10% of samples were run in duplicate with a mean analytical error of 7 ± 8% CV for N.

Nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) were also analyzed according to Standard Methods [*Eaton et al.*, 2005] on the Technicon Nutrient Auto Analyzer. Dissolved organic N (DON) was mathematically calculated as the difference between total and inorganic N. Quality control checks and spikes accompanied each run of NO_3^-, NO_2^-, and NH_4^+ with ~10% of replicates having a mean analytical error of 2 ± 2%, 2 ± 2%, and 6 ± 7% CV, respectively.

DOC samples were acidified to a pH of 2 with 10% HCl prior to analysis via high-temperature combustion on a TOC-VCPN Shimadzu Analyzer as recommended by Benner and Strom [1993]. Samples were run alongside a Consensus Reference Material (CRM; Miami, FL USA) and a subset of samples were run in duplicate with an average analytical error of < 1% CV.

The samples for δ^{13}C-DOC analysis were acidified to a pH < 3 with concentrated H_3PO_4 and oxidized with 1 mL of sodium persulfate oxidizing solution (100 mL H_2O + 4 g $Na_2S_2O_8$ + 200 µL H_3PO_4). The samples were then purged with high purity helium for 5 minutes at 100 mL min^{-1} before they were heated at 100°C for 1 hour to convert DOC to CO_2. The isotopic signature of the resulting CO_2 was analyzed using a GasBench II preparation device connected to a ConFlo IV interface and a Delta V Plus mass spectrometer (Thermo Fisher Scientific). Values were determined using standards prepared over a concentration range that bracketed the samples and had been previously calibrated to IAEA standards (Sucrose, -12.4 ‰; Phthalic Acid, -33.6 ‰) and are reported versus Vienna Pee Dee Belemnite (VPDB) [*Lang et al.*, 2012].

2.7 *Data analysis*

Linear correlations were used to determine the relationships between BOD5 and measured and calculated UBOD as well as chlorophyll-*a*. Due to the nonparametric nature of much of the data, log-log transformations and power functions were implemented to describe the relationships between particulate and dissolved organic C and N, and the independent variable, BOD. Two sample t-tests and analysis of variance (ANOVA) tests were run to compare the difference in BOD and OM concentrations among catchment type and micro-watershed %IC. A matched pair t-test was used to describe the difference between measured and calculated UBOD values as well as the difference in BOD and OM concentrations between paired samples collected along the rising and falling limbs of rain event hydrographs.

Table 2.1 Sample site descriptions and the corresponding drainage basin characteristics.

Site ID	Latitude [°N]	Longitude [°W]	Catchment Class	Urbanization Class [%IC]	Drainage Basin Size [km^2]	Year(s) sampled [yyyy]	Site Description
1	33.33	-79.24	forested stream	none (0.04)	0.29	2018, 2019	drains pine uplands and hardwood swamp
2	33.34	-79.21	forested stream	none (0.08)	2.26	2018, 2019	drains pine uplands and hardwood swamp
3	33.36	-79.19	forested stream	none (0.00)	0.46	2018, 2019	drains pine uplands and hardwood swamp
4	33.36	-79.21	forested stream	none (0.03)	0.49	2019	drains pine uplands and hardwood swamp
5	33.43	-79.16	conveyance	low (4.46)	0.16	2018, 2019	suburban channel; mostly hardwood forest
6a	33.44	-79.16	BMP wetland	low (6.52)	1.72	2018, 2019	natural wetland outfall; low density residential and golf, with ponds; contains 6b drainage basin
6b	33.43	-79.16	conveyance	low (10.31)	0.65	2018, 2019	suburban channel; mostly low density residential; sub-basin of 6a watershed
7	33.47	-79.14	conveyance	low (8.6)	1.65	2018, 2019	suburban channel; mostly low density residential, with ponds
8	33.47	-79.14	conveyance	low (11.53)	0.24	2018, 2019	suburban channel; mostly low density residential and golf, with ponds
9	33.48	-79.10	BMP wetland	medium (19.54)	1.29	2018, 2019	natural wetland outfall; land use is mixed comercial and residental, with ponds
10	33.55	-79.05	conveyance	medium (28.46)	0.13	2018, 2019	suburban channel; land use is medium density residential, no ponds
11	33.55	-79.04	conveyance	high (30.2)	0.41	2018, 2019	suburban channel; land use is medium density residential, some ponds
12	33.56	-79.03	BMP wetland	high (36.16)	0.59	2019	constructed wetland outfall; land use is mixed comercial and residental, with ponds
13	33.56	-79.03	BMP pond	medium (25.49)	1.23	2018, 2019	stormwater pond outfall; mostly medium density residential
14	33.57	-79.05	conveyance	low (11.29)	1.14	2018, 2019	suburban channel; low density residential with many ponds
15	33.59	-79.04	BMP pond	medium (29.90)	0.28	2019	BMP-outfall of single pond serving high density residential development
16	33.59	-79.03	conveyance	high (43.34)	0.47	2018, 2019	suburban channel; mostly commercial/light industrial
17	33.60	-79.02	conveyance	medium (20.23)	1.82	2018, 2019	ditch; mostly high density residential and golf with ponds
18	33.60	-79.02	BMP pond	high (31.95)	0.05	2018, 2019	BMP-outfall of single pond serving high density residential development
19	33.62	-79.03	conveyance	medium (22.10)	0.51	2018, 2019	suburban channel; mainly high density residential with many ponds

% IC is the average percent impervious surface coverage within the drainage basin as determined by watershed delination in ArcGis 10.6.1 and land cover data from Yang et al. [2018]

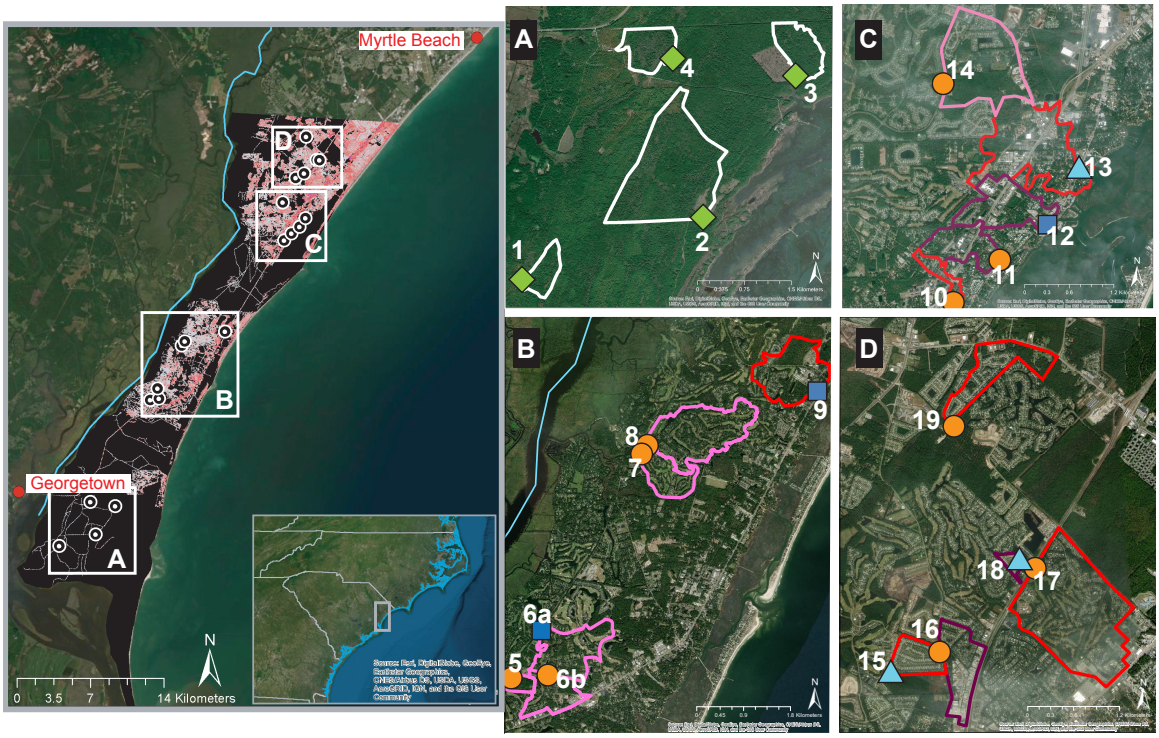

Figure 2.1 The locations of the twenty stormwater catchments are denoted with circles overlain on the National Land Cover Database %IC map (increasing impervious surface from black to dark pink). The blue line marks the Waccamaw River. The inset shows the geographical region within South Carolina containing all sample sites. Subfigures A-D show the individual sampling points of natural streams (sites 1-4; green diamonds), BMP ponds (sites 13, 15, and 18; light blue triangles), BMP wetlands (sites 6a, 9, and 12; dark blue squares), and conveyances (sites 5, 6b, 7-8, 10-11, 13-14, and 16-17; yellow circles). Outlines denote the drainage basin of each site and are color coded to represent the average %IC (white ≤ 1% IC; pink = 1-15% IC; red = 15-30% IC; purple = 30-45% IC).

CHAPTER 3

RESULTS

3.1 *Stormwater BOD concentrations*

A total of 105 samples were collected from urban stormwater BMPs and conveyances during the summers of 2018 and 2019. Collectively these samples produced BOD5 concentrations that ranged from 0.67 to 10.74 mg L^{-1} (Table 3.1). Although stormwater BOD5 was highly variable both among and within sites in response to rain events, they were significantly greater than the BOD5 values of ambient Waccamaw River water collected from May-August of 2018 and 2019 ($p \leq 0.001$; Table 3.1; Figure 3.1; bccmws.coastal.edu/river_gauge). Stormwater BOD5 concentrations were also analogous to the BOD5 concentrations of runoff from pristine forested sites that exhibited similar variability among and within sites (n = 13; Table 3.1; Figure 3.1).

Measured UBOD concentrations that account for respiration of labile OM beyond 5 d were approximately 3 times larger than BOD5 concentrations, but exhibited similar trends (Table 3.1; Figure 3.2). Unlike BOD5, UBOD not only includes degradation of C (CBOD), but also the conversion of organic N compounds and ammonium to nitrate and nitrite (NBOD) (see Appendix A). Of the 77 samples with no nutrients added, NBOD accounted for ~20 to 40% of UBOD after 10 to 20 d. Measured UBOD concentrations ranged from 2.24 mg L^{-1} to 26.52 mg L^{-1} and were similar to the UBOD concentrations of runoff from pristine forested sites (Table 3.1).

The 28 d time course measurements were fit to first order decay kinetics to account for respiration of OM that may occur beyond 28 d (See Equation 2, $R^2 \geq 0.9$). UBOD values for two rising limb samples collected at sites 16 and 19 could not be calculated using first order decay kinetics (exceeded possible iterations), and may be better fit to the sum of two first-order models. Another three rising limb samples collected from sites 4, 13, and 14 were fit to a first order decay ($R^2 \geq 0.9$), but their k-values were low (k ~0.01) and produced unusually high calculated UBOD values (circled in Figure 3.2). The other 72 fitted decay curves produced k coefficients that ranged from 0.03 to 0.89 and UBOD values that ranged from 2.00 mg L^{-1} to 29.10 mg L^{-1} (Table 3.1).

With the removal of the three calculated UBOD outliers, measured and calculated UBOD are both strongly correlated with BOD5 (m = 2.74, $R^2 = 0.94$ and m = 3.10, $R^2 = 0.83$, respectively; Figure 3.2). Calculated UBOD values are significantly higher than that of the measured UBOD ($p < 0.001$), which suggests that degradation continues to occur beyond the 28 d incubation length. Nonetheless the linearity between UBOD and BOD5 indicates that UBOD concentrations exhibit similar patterns described by BOD5 concentrations. Thus, the term BOD is used in the remaining results and discussion to describe trends associated with BOD5 and UBOD.

3.2 *BOD variability with storm event hydrographs and intensity*

In order to differentiate temporal processes in flushing events, BOD samples were collected during rising and falling limbs at each site (n = 29). Rising limb BOD is significantly higher than falling limb BOD ($p \leq 0.01$; Figure 3.3). Storm intensity was examined by monitoring hydrographic height using HOBO loggers and reviewing 24 h rain totals reported on CoCoRaHS (coccorahs.org) and at the North Inlet Winyah Bay

Meteorological Station (cdmo.baruch.sc.edu). There were no clear trends in BOD with hydrographic height or 24 h rain totals when normalized to individual site characteristics, such as catchment type, drainage basin size, and individual catchment shape and size. There was some indication that greater storm intensity may produce higher BOD within an individual site, but the magnitude of BOD increase varied and the number of sites analyzed was too small to be definitive. The number of antecedent dry days prior to each sample event was also determined using HOBO logger, CoCoRaHS, and North Inlet Winyah Bay Meteorological Station datasets. Again no significant trends emerged.

3.3 *Chlorophyll-a concentrations*

Urban stormwater runoff chlorophyll-*a* concentrations varied significantly among sites ($p < 0.01$). Stormwater collected from BMP ponds and conveyances that drain BMP ponds have significantly higher chlorophyll-*a* than conveyances with no pond drainage (both $p < 0.05$; Table 3.2). These chlorophyll-*a* concentrations were also higher than those measured in runoff from pristine sites ($p < 0.05$). When considering all BOD measured, no significant relationship with chlorophyll-*a* was observed. Only when BMP chlorophyll-a data was examined did trends emerge (Figure 3.4). In BMP ponds and wetlands, chlorophyll-*a* explains 75% of BOD variability, with the exclusion of one outlier from site 12 (Figure 3.4; $p < 0.001$).

3.4 *Organic matter concentrations*

Stormwater bulk POC and particulate N (PN) concentrations, which includes algal, macrophyte and terrestrial plant material, were not significantly different between urban and pristine sites (Table 3.2). Stormwater rising limb samples exhibited higher PN and POC concentrations when compared to paired falling limb samples ($p < 0.01$ and $p < 0.05$,

respectively). BOD concentrations were positively correlated to POC and PN concentrations when log-log transformed ($R^2 = 0.30$ for both; $p < 0.001$; Figure 3.5a).

On average, DOC concentrations were greater than POC concentrations in stormwater collected from both altered and pristine catchments (Table 3.2). Stormwater collected from forested sites had consistently higher DOC and DON concentrations compared to urban stormwater runoff ($p < 0.001$; Figure 3.5b, Table 3.2). Despite DOM dominating OM and varying among sites, no strong relationships ($R^2 > 0.2$ and $p > 0.05$) were observed between BOD and DOC or DON.

3.5 ***Organic matter consumption***

Driven by DOC concentrations, total organic carbon (TOC = POC + DOC) in urban stormwater runoff ($\bar{x}$ = 14.7 mg L^{-1}, med=10.8 mg L^{-1}) was significantly lower than in runoff from pristine sites ($\bar{x}$ = 31.6 mg L^{-1}, med=25.4 mg L^{-1}; $p < 0.001$), yet the BOD concentrations were similar. BOD, specifically the carbonaceous fraction (CBOD), only quantified the amount of OM actually catabolized, not the total amount of C present. To evaluate this difference, BOD values were converted to concentrations of TOC consumed and normalized to initial TOC concentrations. Although stormwater from pristine sites had higher concentrations of TOC, a smaller fraction of TOC was ultimately consumed, 12% ± 4%, relative to urban sites (20% ± 9%), across site types and watershed %IC ($p < 0.001$ Figure 3.6).

3.6 ***Stoichiometric ratios and stable isotopes***

To evaluate the difference in OM consumption and potentially different drivers of stormwater BOD in urban versus forested areas, OM was characterized using stoichiometric C:N ratios and stable C isotopes. The POM was fairly heterogeneous across

stormwater structures and watershed %IC with the majority of bulk δ^{13}C-POC values showing a mixture of allochthonous and autochthonous material (Figure 3.7a). A few samples, largely collected from wetland sites (sites 6a and 9), have depleted bulk δ^{13}C-POC ~-37‰ (Figure 3.7a). One sample from a forested site, one from a BMP pond, and three from a conveyance that drains many ponds (sites 2, 13, and 19; Table 2.1) had values that were also depleted in bulk δ^{13}C-POC (Figure 3.7a). The POM at these sites was fairly nitrogen-rich (C:N ~5 to 8; Figure 3.7a).

Unlike POM, DOM stoichiometric ratios and isotopic compositions were distinguishable between stormwater collected from urban versus forested areas (Figure 3.7b). Forested runoff contained nitrogen-poor DOM (C:N > 25) and depleted bulk δ^{13}C-DOC compared to urban runoff (Figure 3.7b). The DOM in urban runoff showed some indication of enrichment in DON and bulk δ^{13}C-DOC values (Figure 3.7b). Further the DOM in urban runoff had characteristics unique to the stormwater site type, but not watershed %IC, as indicated by the clustering of data (Figure 3.7b). On average the DOM released from BMP catchments were more nitrogen-rich and δ^{13}C depleted compared to stormwater conveyances, however the differences are not statistically significant (Figure 3.7b).

Table 3.1 Average stormwater BOD loads and BOD 1st order decay kinetics (k) at each site. BOD5 includes all 108 samples collected in 2018 and 2019. Averages of measured and calculated UBOD as well as k values only contain data from the 77 samples with no nutrient treatment. BOD concentrations and k values of forested catchments, sites 1-4, are similar to urban catchments, sites 5-19.

Site ID	Average BOD loads and 1st order decay kinetics			
	BOD5 [mg L^{-1}]	$UBOD_{measured}$ [mg L^{-1}]	$UBOD_{calculated}$ [mg L^{-1}]	k
1	2.92 ± 1.52	7.69 ± 2.99	8.40 ± 1.73	0.089 ± 0.048
2	3.40 ± 1.55	10.43 ± 3.13	12.15 ± 2.21	0.068 ± 0.025
3	2.15 ± 0.72	8.38 ± 2.67	14.45 ± 11.83	0.053 ± 0.031
4	2.76 ± 0.00	7.71 ± 1.65	8.96 ± 3.24	0.077 ± 0.034
5	3.45 ± 2.68	8.98 ± 7.85	11.06 ± 9.09	0.076 ± 0.033
6a	2.35 ± 0.77	5.48 ± 1.24	5.60 ± 1.10	0.105 ± 0.043
6b	3.18 ± 1.61	8.03 ± 4.36	8.48 ± 4.72	0.098 ± 0.028
7	4.48 ± 3.54	12.23 ± 8.95	13.34 ± 9.13	0.081 ± 0.014
8	2.60 ± 1.30	7.43 ± 3.18	9.95 ± 3.03	0.052 ± 0.019
9	2.33 ± 1.02	7.05 ± 2.18	8.56 ± 2.70	0.064 ± 0.024
10	3.27 ± 1.81	10.01 ± 4.40	11.49 ± 4.75	0.072 ± 0.014
11	2.41 ± 1.58	5.66 ± 1.94	7.83 ± 1.23	0.205 ± 0.212
12	2.41 ± 1.85	5.77 ± 4.77	6.22 ± 4.91	0.281 ± 0.409
13	2.77 ± 1.39	9.02 ± 3.30	16.14 ± 9.72	0.052 ± 0.034
14	2.36 ± 0.93	7.80 ± 3.42	15.98 ± 5.44	0.040 ± 0.039
15	2.90 ± 0.70	10.31 ± 2.11	16.46 ± 1.20	0.038 ± 0.008
16	3.55 ± 1.43	10.95 ± 4.24	14.06 ± 6.71	0.062 ± 0.027
17	4.94 ± 3.10	14.98 ± 8.10	17.04 ± 8.28	0.071 ± 0.015
18	2.20 ± 1.16	6.01 ± 2.61	7.54 ± 3.78	0.068 ± 0.037
19	2.41 ± 1.06	6.65 ± 1.97	8.24 ± 3.04	0.064 ± 0.016
$Mean_{forest}$	2.81	8.55	10.99	0.07
$Median_{forest}$	2.84	8.04	10.56	0.07
SD_{forest}	0.51	1.29	2.84	0.02
$Mean_{urban}$	2.98	8.52	11.13	0.09
$Median_{urban}$	2.69	7.92	10.51	0.07
SD_{urban}	0.81	2.66	3.89	0.06

Table 3.2 Average stormwater dissolved and particulate organic matter concentrations measured at each site in 2018 and 2019. Stormwater from sites 1-4, pristine forested catchments, have significantly higher average DOC and DON concentrations than sites 5-19, urban sites.

Site ID	Average OM concentrations				
	DOC [mg L^{-1}]	DON [mg L^{-1}]	POC [mg L^{-1}]	PN [mg L^{-1}]	Chl-*a* [µg L^{-1}]
1	20.3 ± 3.6	0.61 ± 0.10	1.9 ± 2.0	0.23 ± 0.22	3.6 ± 1.6
2	32.6 ± 18.7	1.01 ± 0.45	5.7 ± 6.0	0.57 ± 0.46	12.1 ± 9.5
3	15.1 ± 2.9	0.65 ± 0.30	2.7 ± 3.5	0.30 ± 0.36	1.9 ± 0.5
4	24.5 ± 6.9	0.75 ± 0.43	1.0 ± 1.1	0.16 ± 0.18	7.8 ± 2.5
5	12.7 ± 2.8	0.48 ± 0.07	2.5 ± 3.2	0.33 ± 0.43	3.6 ± 3.9
6a	8.0 ± 0.7	0.44 ± 0.12	0.9 ± 0.6	0.20 ± 0.12	10.3 ± 3.5
6b	9.2 ± 2.2	0.41 ± 0.08	5.4 ± 7.7	0.47 ± 0.50	16.6 ± 15.7
7	9.1 ± 0.7	0.53 ± 0.06	9.0 ± 12.5	0.80 ± 0.91	22.1 ± 25.9 *
8	9.0 ± 1.5	0.52 ± 0.06	4.4 ± 7.6	0.38 ± 0.51	38.4 ± 22.2 *
9	8.2 ± 1.3	0.41 ± 0.03	0.6 ± 0.4	0.12 ± 0.09	31.5 ± 13.6 *
10	12.4 ± 1.6	0.51 ± 0.08	2.9 ± 4.7	0.29 ± 0.30	10.6 ± 7.5
11	8.2 ± 1.0	0.48 ± 0.04	5.2 ± 9.0	0.49 ± 0.63	8.0 ± 1.0
12	10.0 ± 0.6	0.41 ± 0.05	4.0 ± 5.4	0.32 ± 0.41	5.1 ± 5.5
13	9.4 ± 1.5	0.48 ± 0.06	1.9 ± 0.9	0.41 ± 0.25	38.1 ± 30.5 *
14	22.0 ± 8.2	0.51 ± 0.18	7.5 ± 4.0	0.19 ± 0.04	2.7 [a] ± 0.0
15	7.9 ± 0.3	0.55 ± 0.07	1.7 [a] ± 0.0	1.29 ± 0.48	34.4 ± 32.6 *
16	8.1 ± 1.8	0.47 ± 0.06	3.5 ± 2.6	0.36 ± 0.19	13.2 ± 12.5
17	11.3 ± 4.3	0.61 ± 0.20	5.3 ± 6.4	0.77 ± 0.65	17.0 ± 5.1
18	6.9 ± 1.3	0.48 ± 0.04	5.8 ± 11.8	0.26 ± 0.12	33.5 ± 14.2 *
19	7.1 ± 1.0	0.47 ± 0.08	1.7 ± 0.9	0.29 ± 0.12	26.0 ± 14.0 *
Mean $_{forest}$	23.1 *	0.75 *	2.8	0.31	6.35
Median $_{forest}$	22.4 *	0.70 *	2.3	0.26	5.70
SD $_{forest}$	7.4	0.18	2.0	0.18	4.56
Mean $_{urban}$	10.0	0.48	3.7	0.44	20.6 *
Median $_{urban}$	9.0	0.48	2.9	0.34	17.0 *
SD $_{urban}$	3.62	0.05	2.3	0.29	12.3

[a] Not an average value; two samples at site with one outlier removed

*Significantly higher values

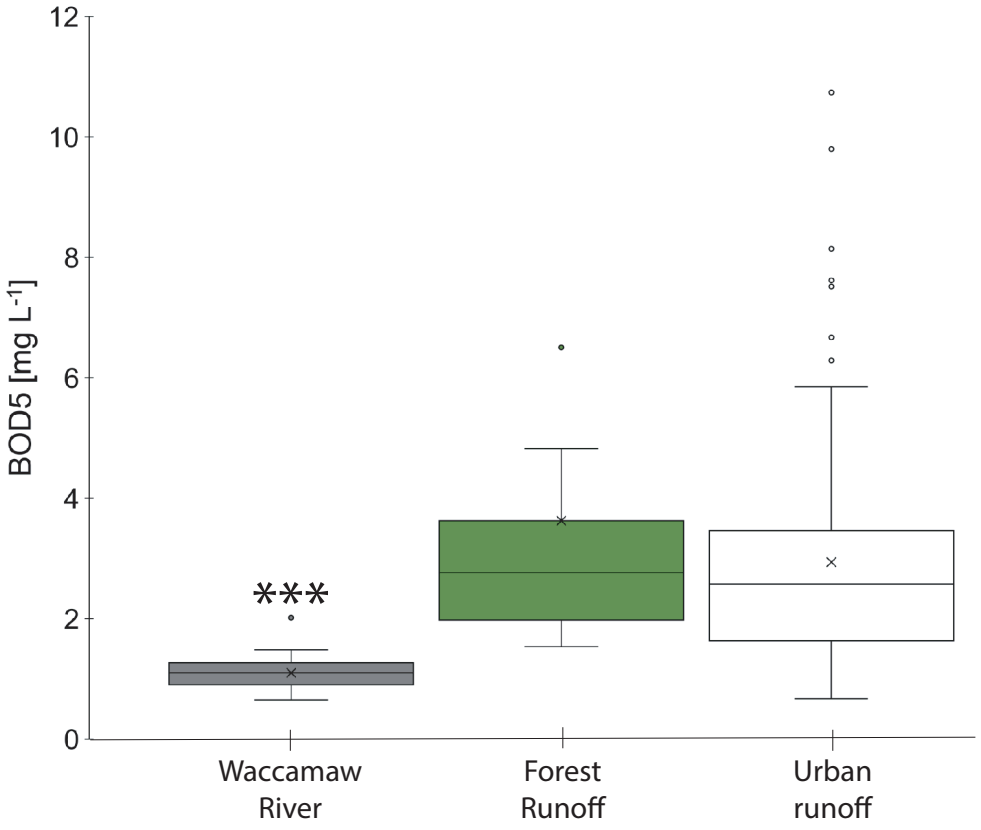

Figure 3.1 The plot compares the stormwater BOD5 loads from pristine forested sites (middle box; one outlier not on scale) and urban catchments (right box) with BOD5 values of ambient Waccamaw River water (left box) measured in the summers of 2018 and 2019 (http://bccmws.coastal.edu/river_gauge/). The asterisk marks the significant difference between ambient river BOD and stormwater BOD.

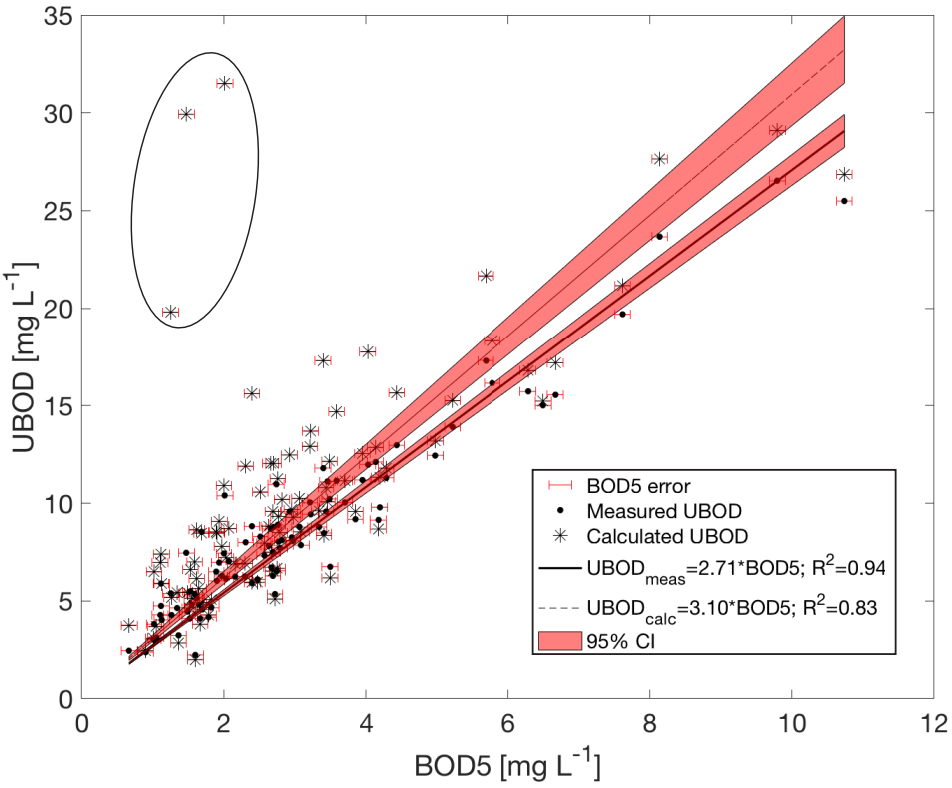

Figure 3.2 All 2018 and 2019 measured UBOD loads (black circles) and calculated UBOD loads (gray asterisks) are plotted against BOD5 loads. A strong linear relationship (R^2>0.94; $p < 0.001$) exists between measured UBOD and BOD5 values as well as calculated UBOD and BOD5 ($R^2 > 0.83$; $p < 0.001$).

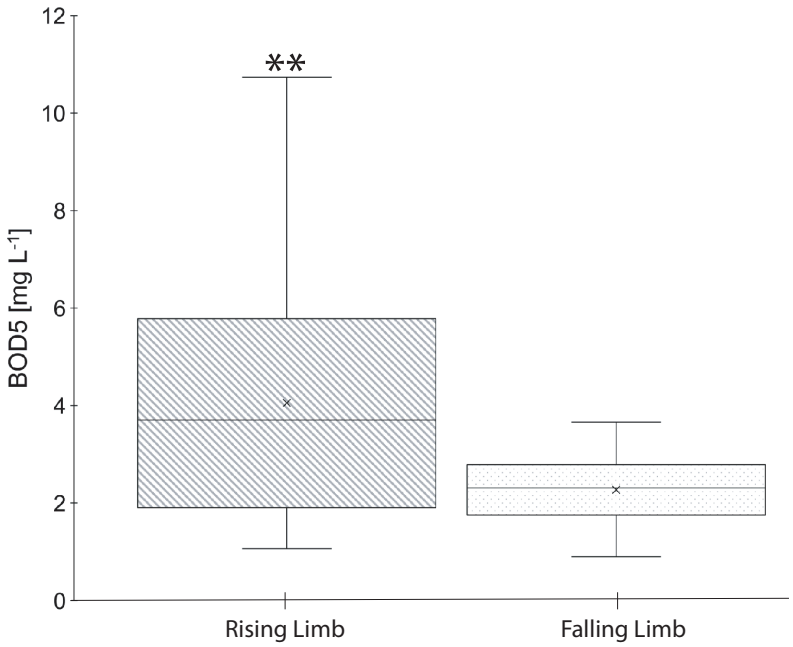

Figure 3.3 Differences in stormwater BOD5 values measured on paired samples collected along the rising (left box) and falling limb (right box) of rain event hydrographs in 16 urbanized catchments. The asterisks above the box denotes a significant difference ($p < 0.01$).

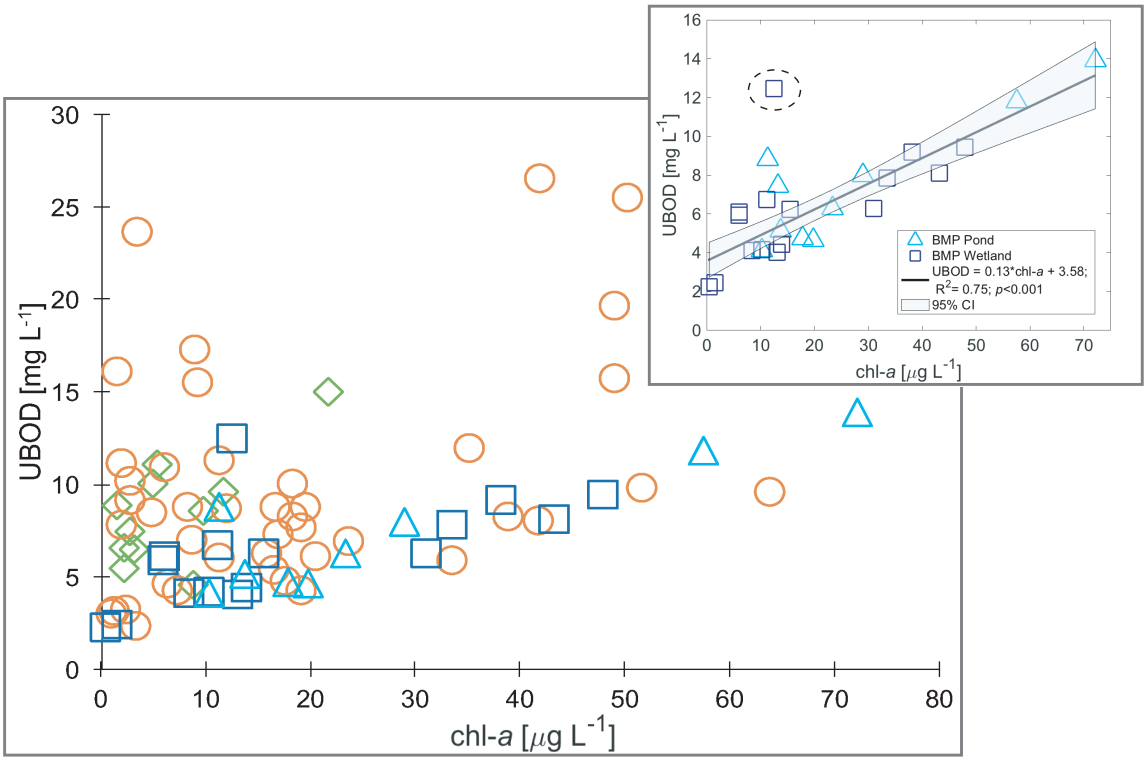

Figure 3.4 UBOD concentrations versus chlorophyll-*a* concentrations. No significant relationship when evaluating the full dataset (forested streams=green diamonds, urban conveyances=orange circles, BMP-ponds=light blue diamonds, BMP-wetlands=blue squares). The inset shows a strong relationship between UBOD and chlorophyll-*a* measured in runoff from BMP sites (m = 0.13, $R^2 = 0.75$).

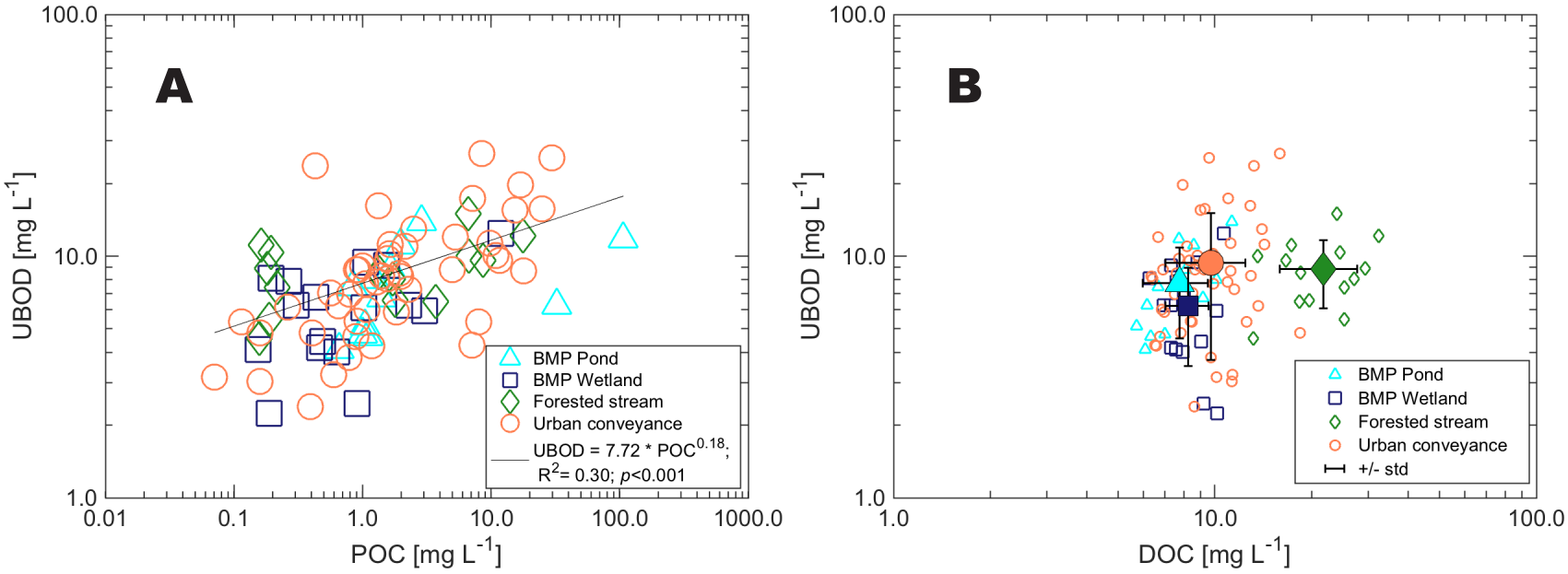

Figure 3.5 Measured UBOD versus the concentrations of (**A**) POC and (**B**) DOC. The data is displayed by catchment type: forested streams (green diamonds), BMP wetlands (dark blue squares), BMP ponds (light blue triangles), and urban conveyances (orange circles). Both plots are on log-log scales. Plot A shows the correlation between UBOD and POC expressed as a power function ($R^2 = 0.30$ and $p < 0.001$). Plot B depicts the average DOC and UBOD concentrations for each stormwater site type as the larger filled markers and the standard deviations represented as error bars. Individual sample error is smaller than the individual sample symbols.

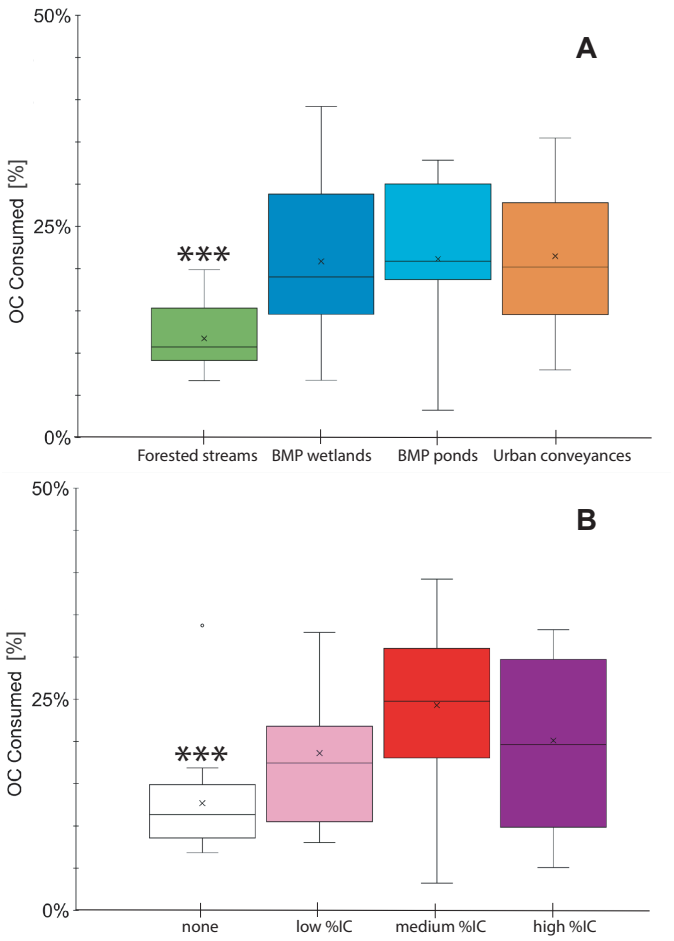

Figure 3.6 Box and whisker plots show the range in normalized organic carbon (OC) ultimately consumed as derived from the UBOD values converted to moles of carbon (1.2 O_2:1 C) divided by the moles of total organic carbon (measured POC+DOC) based on site type (**A**) and drainage basin %IC (**B**). The asterisks denote a significant difference ($p < 0.001$).

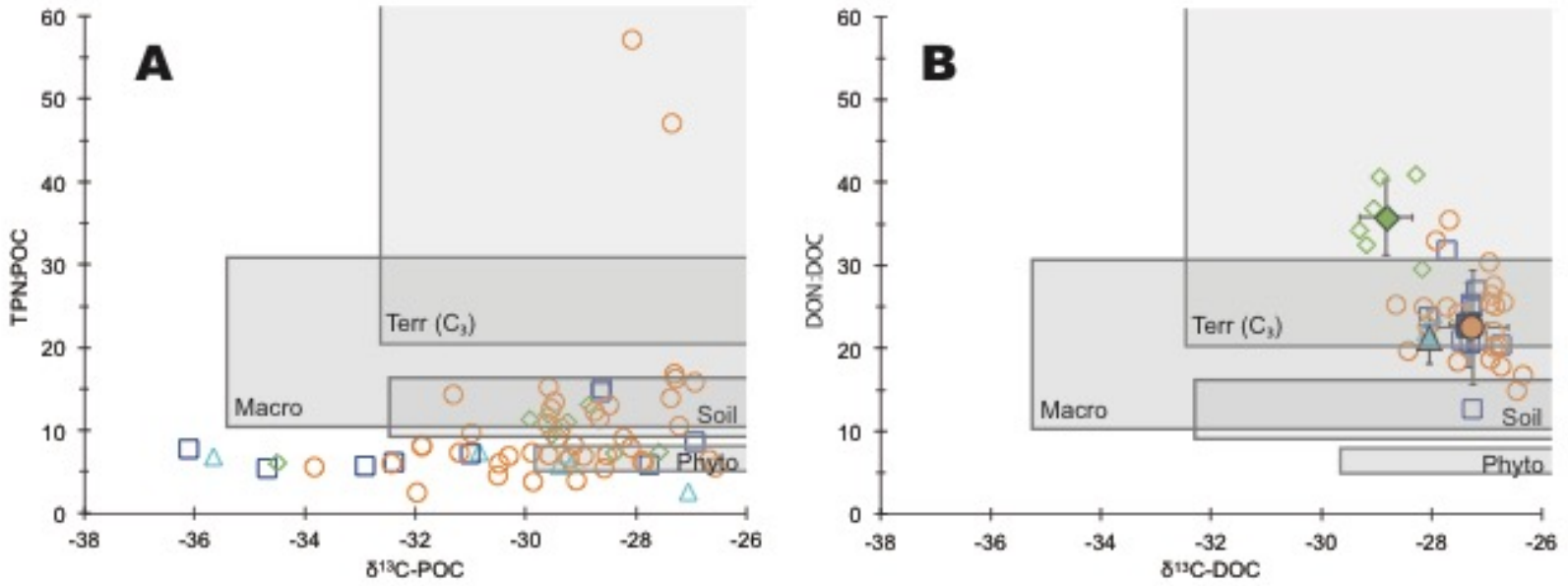

Figure 3.7 Stable isotopic fractions of (**A**) POC and (**B**) DOC versus molar ratios of C:N. Data is displayed by site type: natural streams (green diamonds), BMP wetlands (dark blue squares), BMP ponds (light blue triangles), and urban conveyances (orange circles). The boxes illustrate the compositional ranges derived from previously published studies (e.g., *Goñi and Thomas*, 2000; *Kendall et al.*, 2001; *Goñi et al.*, 2003). The compositional ranges include Terr (C_3), terrestrial C_3 vascular plants; Phyto, freshwater algal material; Soil, soil OM; and Macro, aquatic macrophytes. Larger filled markers in plot **B** represent average values with the standard deviation represented as error bars.

CHAPTER 4

DISCUSSION

4.1 *Stormwater BOD5 and UBOD*

The lack of information on stormwater OM lability and degradation kinetics has hindered the current understanding of coastal DO impairment. Our BOD data provides much needed insight. First and foremost, microbial respiration of OM in stormwater runoff continues beyond 5 d and was detectable over 28 d. However, BOD5 and UBOD concentrations exhibit similar patterns in variability among and within sites, with the short-term BOD scalable to UBOD using a conversion factor of approximately 3 (Figure 3.2). Like point source pollutants [*Eaton et al.*, 2005], the consumption of oxygen demanding substances in stormwater primarily follow first order decay ($R^2 > 0.9$), despite a contribution of BOD from nitrification around 10 to 20 d (20 to 40%; see Appendix A). Currently regulatory agencies rely on conversion factors determined by 'best professional judgement' that may under or overestimate BOD. Our derived conversion factor provides a standardized value that may improve stormwater UBOD concentration estimates that are incorporated into NPDES permits and TMDL plans in SC and throughout the southeast.

4.2 *Stormwater BOD characteristics*

Urban stormwater runoff has BOD concentrations that substantially exceed ambient BOD concentrations in the Waccamaw River (Figure 3.1; $p \leq 0.001$). This suggests stormwater is a significant source of BOD that contributes to low DO in the

coastal receiving waters of this region. Although, stormwater BOD concentrations are much lower than point source BOD concentrations [*US EPA*, 1995; *SCDHEC*, 1999; *U.S. EPA*, 2013]. The BOD concentrations measured across the SC coastal plain were analogous to concentrations previously reported in urban and suburban lotic systems in North Carolina during a mix of weather conditions, including high BOD after rain events [*Mallin et al.*, 2009]. The results presented here show that storm events produce broadly similar BOD concentrations regardless of the type of stormwater structure or degree of urbanization (as %IC) in the catchment area. Concentrations of BOD do, however, vary greatly among and within sites.

Urban runoff BOD concentrations vary among sites in response to rain events. Yet similar BOD statistical midpoints and distributions are observed across site classifications and the gradient of drainage basin %IC, including BOD concentrations of runoff from pristine forested areas (Table 3.1; Figure 3.1). The similarity refutes the hypothesis that the conversion of natural landscapes to landscapes with high %IC increases BOD concentrations in stormwater runoff. Mallin et al. [2009], for example, found significant correlations between ambient BOD in streams and drainage basin %IC in North Carolina. Results of the present study suggest that the BOD of stormwater runoff is high regardless of land cover and land use in coastal SC. This difference is likely a reflection of the naturally high organic content of the forested wetlands within the forested catchments sampled in this study [*Wahl et al.*, 1997] relative to those of the Mallin et al. [2009] study.

The high OM content in forested wetlands, the predominate type of pre-developed land cover in the SC coastal plain, causes the Waccamaw River to have naturally low DO (13 y daily summer average = 4.4 mg L^{-1}; waterdata.usgs.gov, site 02110802). Therefore

it is not entirely surprising that runoff from pristine forested sites in this region had high BOD concentrations that were analogous to the BOD concentrations of urban runoff, but it does not reconcile the further decline in DO levels in the Waccamaw River over the past several decades [*SCDHEC*, 1999, 2018]. Given the extensive network of stormwater flow paths displayed in community watershed plans [*Waccamaw Regional Council of Governments*, 2014] and the level of %IC in these watersheds (~5 to 43%; Table 2.1), an increased volume of stormwater discharge [*Smith et al.*, 2013; *Meierdiercks et al.*, 2017] that could result in subsequently higher total export of OM [*Wahl et al.*, 1997] may help resolve this disconnect between stormwater BOD concentrations and worsening downstream DO impairment.

4.3 *Hydrologic influences and flushing effects*

Hydrologic influences were shown to effect stormwater pollutant concentrations. As hypothesized, the stormwater BOD concentrations of rising limb samples were significantly greater than BOD concentrations of falling limb samples (Figure 3.3). These rising limb pollutant concentrations were accompanied by the highest POC and PN concentrations, that sometimes surpassed DOM concentrations. These findings support the idea of a first flush effect where the mobilization of larger quantities of OM, specifically POM, occur with the initial rise in the hydrograph or initial pulse of stormwater runoff [*Clark et al.*, 1980; *Walsh et al.*, 2005; *Smith and Kaushal*, 2015]. As the hydrograph begins to fall and the rate of flow slows to baseflow, less pollutants are carried downstream [*Walsh et al.*, 2005]. This finding helps explain some of the within site BOD variability observed, although the range in both rising and falling limb BOD values at a single site were still quite large.

It is generally accepted that OM fluxes scale with storm intensity [*Raymond and Oh*, 2007; *Raymond and Spencer*, 2015], but no overall trends emerged between hydrographic height or 24 h rain totals and BOD concentrations. Other weather and anthropogenic influences such as antecedent dry conditions that may promote outdoor activities (i.e., application of fertilizers, pet waste, construction work, etc.) also likely account for individual site variability, yet no trends were observed between BOD concentrations and the number of antecedent dry days prior to each sample event. Further investigation with more repeat sampling at individual locations is required to understand the scale at which different storm intensities and various antecedent weather conditions impact stormwater BOD concentrations.

Stormwater hydrologic regimes also differed with site type. BMPs are designed to mitigate first flush effects by reducing the rate of stormwater flow downstream to mimic natural aquatic systems [*Leopold*, 1968; *Clark et al.*, 1980]. Peak height was reduced at BMP sites, as evident in HOBO logger data (data not shown), but BOD, DOM, and POM, particularly chlorophyll-*a* containing POM, concentrations were not reduced. High chlorophyll-*a* was even observed downstream in the conveyances draining detention ponds. Other studies have also documented high levels of OM in BMP outflow [*Mallin et al.*, 2002] and stormwater ponds were shown to be ineffective at sequestering autochthonous POM [*Schroer et al.*, 2018]. Therefore, hydrological alterations need to be considered in the context of catchment biogeochemistry to ensure water quality improvement.

4.4 *POM quantity and quality as drivers of stormwater BOD*

POM concentrations in stormwater runoff exceed the range of POC and PN concentrations previously reported in the surface waters of the Waccamaw River and Winyah Bay [*Goñi et al.*, 2003]. Stormwater is widely accepted as a primary transport mechanism of POM due to the pulse of high flow capable of mobilizing larger material, especially during the first flush. Historically, suspended POM was thought to comprise a small fraction of the largely refractory OM in aquatic systems with average turnover times on the order of weeks to years [*Ittekkot*, 1988; *Hedges et al.*, 1997; *Webster et al.*, 1999]. In contrast, the positive relationship between stormwater POM concentrations (Figure 3.5a), especially chlorophyll-*a* concentrations (Figure 3.4), suggests that POM is a significant driver of BOD. These results support more recent work that has reassessed the importance of POM, especially fresh and labile compounds, to aquatic microbial respiration [*Battin et al.*, 2008; *Tremblay and Gagné*, 2009; *Richardson et al.*, 2013]. For example, Richardson et. al. [2013] found that bioavailable suspended POM was consumed rapidly (~10 d) and locally (within 1.5 km). The turnover time may be even more rapid (~7 d) when POM contains large quantities of algal material, as indicated by high chlorophyll-*a* [*Richardson et al.*, 2013], such as that discharged from pond and wetland BMPs (Table 3.2; Figure 3.4).

The lability of POM carried by stormwater runoff is further supported by the isotopic composition and molar ratios of POM. Stormwater POM is largely a heterogeneous mixture of allochthonous and autochthonous plant material, but is fairly nitrogen-rich compared to DOM (Figure 3.7). Replete nitrogen compounds have been shown to be more labile than depleted samples [*del Giorgio and Cole*, 1998]. Low C:N

ratios may be indicative of a greater contribution of freshwater phytoplankton material or of microbial alterations of terrestrial OM [*Rice and Hanson*, 1984; *Kendall et al.*, 2001; *Goñi et al.*, 2003]. The former most likely explains the low C:N in BMP sites and sites draining BMP ponds, since those samples also exhibit high chlorophyll-*a* concentrations that were similar to chlorophyll-*a* concentrations measured in other SC stormwater ponds [*Bricker et al.*, 2003; *Drescher et al.*, 2007]. The latter might better explain the nitrogen-rich POM released in runoff from forested sites. The few sites with depleted δ^{13}C-POC and low C:N, especially those from BMP wetlands, were likely indicative of a mixture of aquatic macrophytes and algal material [*Keough et al.*, 1998; *Kendall et al.*, 2001]. Emergent wetland species that often have depleted C isotopic signatures (as low as -36‰) relative to submerged vegetation and floating leaf plants [*Keough et al.*, 1998], were present in large quantities at all wetland sites. Overall, these proxies for lability support the notion that POM is a significant labile fraction that can fuel stormwater BOD.

4.5 DOM lability and contribution to stormwater BOD

The microbial respiration of the POM fraction cannot alone explain the magnitude of stormwater BOD concentrations in most samples (POM explained 30% of variability in BOD and initial POM concentrations could comprise on median ~60% of the BOD). Some amount of DOM, the primary substrate for microbial respiration, must also be consumed. Consistent with DOM being one of the largest pools of C on Earth [*Carlson and Hansell*, 2014], DOM tended to be the larger pool of OM in stormwater runoff. Although forested runoff contained significantly higher DOM concentrations than the urban sites ($p < 0.001$; Table 3.2). Others have also reported decreases in lotic DOC and DON concentrations associated with urbanization in the SC coastal plain, but noted these urban DOM

concentrations scale with increased flow to similar annual DOM exports as forests [*Wahl et al.*, 1997; *Tufford et al.*, 2003]. BOD concentrations, however, were not correlated with DOM concentrations.

DOM is often quite degraded and recalcitrant [*Raymond et al.*, 2004] with only a very small labile fraction of DOM rapidly cycled [*Smith and Kaushal*, 2015]. The bulk C isotopic compositions and especially the high C:N ratios of DOM in urban runoff suggest a fairly refractory pool of terrestrial C [*Kendall et al.*, 2001]. However, the slight enrichment in δ^{13}C-DOC and low C:N reported in urban stormwater runoff compared to forested runoff, especially from BMPs and conveyances draining BMPs, suggests it may have a contribution of algal or macrophyte material [*Kendall et al.*, 2001], which are generally considered to be more labile [*del Giorgio and Cole*, 1998; *Benner*, 2007]. Further urban stormwater runoff exhibited significantly higher TOC consumption rates over the course of the 28 d incubation compared to runoff from forested sites ($p < 0.001$), but average TOC consumption rates are < 20%. Results presented here, therefore, support the growing body of literature that suggest urbanization and the accompanying alterations to headwaters are enhancing the bioavailability of OM in aquatic systems [*Wiegner et al.*, 2006; *Kaushal et al.*, 2014; *Lu et al.*, 2014; *Wallace et al.*, 2014].

4.6 ***Management recommendations***

Stormwater runoff BOD concentrations are significant compared to ambient river concentrations, and should thus be considered as factors when managing for DO impairment in downstream systems. Efforts to define land use characteristics are not important when estimating BOD concentrations in this region, although land use is likely an important factor altering the hydrology of stormwater flow that may significantly scale

BOD loads and OM exports. Current modern stormwater infrastructure, including BMPs based on comparisons with concentrations measured in stormwater piped and ditched conveyances, is largely ineffective at reducing BOD concentrations. Stormwater BMPs, especially detention ponds, actually release significant amounts of fresh labile, autochthonous OM. In fact, urbanization and the associated headwater alterations are changing the source and composition of POM and increasing the fraction of potentially labile DOM that fuel microbial respiration within inland and coastal waters. Stormwater BOD concentrations, however, are relatively unaffected by these changes, but the changes may have a significant impact on the global carbon cycle. Since BOD concentrations remain relatively constant across stormwater control structures and degree of urbanization (as %IC) in the catchment, and BOD decay kinetics follow a first order model, a standardized conversion factor of 3 can be adopted for regulatory modeling purposes. In order to effectively implement stormwater regulations and mitigate costal DO impairment, local and state agencies should aim to reduce stormwater POM exports from land and from within stormwater infrastructure, that are shown to drive BOD.

REFERENCES

Arar, E. J., and G. B. Collins (1997), Method 445.0: Chlorophyll A, *U.S. Environ. Prot. Agency*, (September), 1–22.

Battin, T. J., L. A. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, A. I. Packman, J. D. Newbold, and F. Sabater (2008), Biophysical controls on organic carbon fluxes in fluvial networks, *Nat. Geosci.*, doi:10.1038/ngeo101.

Benner, R. (2003), Molecular Indicators of the Bioavailability of Dissolved Organic Matter, in *Aquatic Ecosystems Interactivity of Dissolved Organic Matter*, edited by S. Findlay and R. Sinsabaugh, pp. 121–135.

Benner, R. (2007), Chemical Composition and Reactivity, in *Biogeochemistry of Marine Dissolved Organic Matter*.

Berggren, M., J. F. Lapierre, and P. A. Del Giorgio (2012), Magnitude and regulation of bacterioplankton respiratory quotient across freshwater environmental gradients, *ISME J.*, *6*(5), 984–993, doi:10.1038/ismej.2011.157.

Bhattacharya, R., and C. L. Osburn (2020), Spatial patterns in dissolved organic matter composition controlled by watershed characteristics in a coastal river network: The Neuse River Basin, USA, *Water Res.*, *169*, 115248, doi:10.1016/j.watres.2019.115248

Breitburg, D. et al. (2018), Declining oxygen in the global ocean and coastal waters, *Science (80-.).*, doi:10.1126/science.aam7240.

Bricker, S. B., J. G. Ferreira, and T. Simas (2003), An integrated methodology for assessment of estuarine trophic status, *Ecol. Modell.*, doi:10.1016/S0304-3800(03)00199-6.

Carlson, C. A., and D. A. Hansell (2014), DOM Sources, Sinks, Reactivity, and Budgets, in *Biogeochemistry of Marine Dissolved Organic Matter: Second Edition*.

Clark, J., T. Dunne, and L. B. Leopold (1980), Water in Environmental Planning, *J. Wildl. Manage.*, doi:10.2307/3808399.

Delzer, G. C., and S. W. Mckenzie (2003), Five-day biochemical oxygen demand, in *TWRI Book 9*, vol. 3.

Díaz, F. J., A. T. Chow, A. T. O'Geen, R. A. Dahlgren, and P. K. Wong (2008), Restored wetlands as a source of disinfection byproduct precursors, *Environ. Sci. Technol.*, doi:10.1021/es800781n.

Diaz, R. J., and R. Rosenberg (1995), Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna, *Oceanogr. Mar. Biol. an Annu. Rev. Vol. 33*, (May 2014), 245–303.

Diaz, R. J., and R. Rosenberg (2008), Spreading dead zones and consequences for marine ecosystems, *Science (80-.).*, doi:10.1126/science.1156401.

Doney, S. C. (2010), The growing human footprint on coastal and open-Ocean biogeochemistry, *Science (80-.).*, doi:10.1126/science.1185198.

Downing, J. A. (2010), Emerging global role of small lakes and ponds: Little things mean a lot, *Limnetica*.

Drescher, S. R., M. J. Messersmith, and D. M. Sanger (2007), FINAL REPORT FOR TWO OCRM STUDIES : Stormwater pond baseline and efficiency, , (June), 1–11.

Eaton, A. D., L. S. Clesceri, and A. E. Greenbaerg (2005), Standard Methods for Examination of Water and Wastewater (Standard Methods for the Examination of Water and Wastewater)., *Am. Public Heal. Assoc. Washington, DC, USA*, *21 Ed.*, doi:ISBN 9780875532356.

Emerson, C. H., C. Welty, and R. G. Traver (2005), Watershed-scale evaluation of a system of storm water detention basins, *J. Hydrol. Eng.*, doi:10.1061/(ASCE)1084-0699(2005)10:3(237).

Georgetown County (2015), *Georgetown County Stormwater Management Plan (SWMP)*, Georgetown, SC.

del Giorgio, P. A., and J. J. Cole (1998), Bacterial Growth Efficiency in Natural Aquatic Systems, *Annu. Rev. Ecol. Syst.*, *29*(1), 503–541, doi:10.1146/annurev.ecolsys.29.1.503.

del Giorgio, P. A. del, and J. Davis (2003), Patterns in Dissolved Organic Matter Lability and Consumption across Aquatic Ecosystems, in *Aquatic Ecosystems Interactivity of Dissolved Organic Matter*, edited by S. Findlay and R. Sinsabaugh, pp. 400–420.

Goñi, M. A., and K. A. Thomas (2000), Sources and transformations of organic matter in surface soils and sediments from a tidal estuary (North Inlet, South Carolina, USA), *Estuaries*, *23*(4), 548–564, doi:10.2307/1353145.

Goñi, M. A., M. J. Teixeira, and D. W. Perkeya (2003), Sources and distribution of organic matter in a river-dominated estuary (Winyah Bay, SC, USA), *Estuar. Coast. Shelf Sci.*, doi:10.1016/S0272-7714(03)00008-8.

Harder, S. V., D. M. Amatya, T. J. Callahan, C. C. Trettin, and J. Hakkila (2007), Hydrology and water budget for a forested Atlantic Coastal Plain watershed, South Carolina, *J. Am. Water Resour. Assoc.*, *43*(3), 563–575, doi:10.1111/j.1752-1688.2007.00035.x.

Hedges, J. I., R. G. Keil, and R. Benner (1997), What happens to terrestrial organic matter in the ocean?, *Org. Geochem.*, *27*(5–6), 195–212, doi:10.1016/S0146-6380(97)00066-1.

Horry County (2014), *Horry County Stormwater Management Plan (SWMP)*, Conway, SC.

Hosen, J. D., O. T. McDonough, C. M. Febria, and M. A. Palmer (2014), Dissolved organic matter quality and bioavailability changes across an urbanization gradient in headwater streams, *Environ. Sci. Technol.*, *48*(14), 7817–7824, doi:10.1021/es501422z.

Hudson, N., A. Baker, D. Ward, D. M. Reynolds, C. Brunsdon, C. Carliell-Marquet, and S. Browning (2008), Can fluorescence spectrometry be used as a surrogate for the Biochemical Oxygen Demand (BOD) test in water quality assessment? An example from South West England, *Sci. Total Environ.*, *391*(1), 149–158, doi:10.1016/j.scitotenv.2007.10.054.

Ittekkot, V. (1988), Global trends in the nature of organic matter in river suspensions, *Nature*, *332*(6163), 436–438, doi:10.1038/332436a0.

Kaushal, S. S., P. M. Mayer, P. G. Vidon, R. M. Smith, M. J. Pennino, T. A. Newcomer, S. Duan, C. Welty, and K. T. Belt (2014), Land use and climate variability amplify carbon, nutrient, and contaminant pulses: A review with management implications, *J. Am. Water Resour. Assoc.*, doi:10.1111/jawr.12204.

Kendall, C., S. R. Silva, and V. J. Kelly (2001), Carbon and nitrogen isotopic compositions of particulate organic matter in four large river systems across the United States, *Hydrol. Process.*, *15*(7), 1301–1346, doi:10.1002/hyp.216.

Keough, J. R., C. A. Hagley, E. Ruzycki, and M. Sierszen (1998), δ13C composition of primary producers and role of detritus in a freshwater coastal ecosystem, *Limnol. Oceanogr.*, *43*(4), 734–740, doi:10.4319/lo.1998.43.4.0734.

Khamis, K., C. Bradley, and D. M. Hannah (2017), Understanding dissolved organic matter dynamics in urban catchments: insights from *in situ* fluorescence sensor technology, *Wiley Interdiscip. Rev. Water*, *5*(February), e1259, doi:10.1002/wat2.1259.

Lang, S. Q., S. M. Bernasconi, and G. L. Früh-Green (2012), Stable isotope analysis of organic carbon in small (μg C) samples and dissolved organic matter using a GasBench preparation device, *Rapid Commun. Mass Spectrom.*, *26*(1), 9–16, doi:10.1002/rcm.5287.

Leopold, L. (1968), Hydrology for Urban Land Planning - A Guidebook on the Hydrologic Effects of Urban Land Use, *Geol. Surv. Circ.*

Lewitus, A. J., L. M. Brock, M. K. Burke, K. A. DeMattio, and S. B. Wilde (2008), Lagoonal stormwater detention ponds as promoters of harmful algal blooms and eutrophication along the South Carolina coast, *Harmful Algae*, doi:10.1016/j.hal.2008.08.012.

Lu, Y. H., J. E. Bauer, E. A. Canuel, R. M. Chambers, Y. Yamashita, R. Jaffé, and A. Barrett (2014), Effects of land use on sources and ages of inorganic and organic carbon in temperate headwater streams, *Biogeochemistry*, *119*(1–3), 275–292, doi:10.1007/s10533-014-9965-2.

Lusk, M. G., and G. S. Toor (2016), Dissolved organic nitrogen in urban streams: Biodegradability and molecular composition studies, *Water Res.*, *96*, 225–235, doi:10.1016/j.watres.2016.03.060.

Mallin, M. A., S. H. Ensign, T. L. Wheeler, and D. B. Mayes (2002), Pollutant Removal Efficacy of Three Wet Detention Ponds Michael, *J. Environ. Qual.*, doi:10.2134/jeq2002.6540.

Mallin, M. A., V. L. Johnson, and S. H. Ensign (2009), Comparative impacts of stormwater runoff on water quality of an urban, a suburban, and a rural stream, *Environ. Monit. Assess.*, *159*(1–4), 475–491, doi:10.1007/s10661-008-0644-4.

McCuen, R. H. (1979), Downstream effects of stormwater management basins., *J. Hydraul. Div. ASCE*.

McElmurry, S. P., D. T. Long, and T. C. Voice (2014), Stormwater Dissolved Organic Matter: Influence of Land Cover and Environmental Factors, *Environ. Sci. Technol.*, *48*(1), 45–53, doi:10.1021/es402664t.

Meierdiercks, K. L., M. B. Kolozsvary, K. P. Rhoads, M. Golden, and N. F. McCloskey (2017), The role of land surface versus drainage network characteristics in controlling water quality and quantity in a small urban watershed, *Hydrol. Process.*, *31*(24), 4384–4397, doi:10.1002/hyp.11367.

O'Geen, A. T., R. Budd, J. Gan, J. J. Maynard, S. J. Parikh, and R. A. Dahlgren (2010), *Mitigating nonpoint source pollution in agriculture with constructed and restored wetlands*, 1st ed., Elsevier Inc.

Obernosterer, I., and R. Benner (2004), Competition between biological and photochemical processes in the mineralization of dissolved organic carbon, *Limnol. Oceanogr.*, doi:10.4319/lo.2004.49.1.0117.

Petrone, K. C., J. B. Fellman, E. Hood, M. J. Donn, and P. F. Grierson (2011), The origin and function of dissolved organic matter in agro-urban coastal streams, *J. Geophys. Res. Biogeosciences*, *116*(1), doi:10.1029/2010JG001537.

Raymond, P. A., and N. H. Oh (2007), An empirical study of climatic controls on riverine C export from three major U.S. watersheds, *Global Biogeochem. Cycles*, *21*(2), 1–9, doi:10.1029/2006GB002783.

Raymond, P. A., and R. G. M. Spencer (2015), Riverine DOM, in *Biogeochemistry of Marine Dissolved Organic Matter: Second Edition*.

Raymond, P. A., J. E. Bauer, N. F. Caraco, J. J. Cole, B. Longworth, and S. T. Petsch (2004), Controls on the variability of organic matter and dissolved inorganic carbon ages in northeast US rivers, in *Marine Chemistry*.

Rice, D. L., and R. B. Hanson (1984), A kinetic model for detritus nitrogen: role of the associated bacteria in nitrogen accumulation., *Bull. Mar. Sci.*, *35*(3), 326–340.

Richardson, D. C., J. D. Newbold, A. K. Aufdenkampe, P. G. Taylor, and L. A. Kaplan (2013), Measuring heterotrophic respiration rates of suspended particulate organic carbon from stream ecosystems, *Limnol. Oceanogr. Methods*, *11*(MAY), 247–261, doi:10.4319/lom.2013.11.247.

SCDHEC (1999), State of South Carolina Administrative Record TMDL Submittal for Waccamaw River and Atlantic Intracoastal Waterway Biochemical Oxygen Demand, , 3–21.

SCDHEC (2014), R.61-68, Water Classifications and Standards, , *2014*(803).

SCDHEC (2018), *The State of South Carolina' s 2018 Integrated Report (IR) Part I : Listing of Impaired Waters*.

Schroer, W. F., C. R. Benitez-Nelson, E. M. Smith, and L. A. Ziolkowski (2018), Drivers of Sediment Accumulation and Nutrient Burial in Coastal Stormwater Detention Ponds, South Carolina, USA, *Ecosystems*, 1–21, doi:10.1007/s10021-017-0207-z.

Schueler, T. R. (1994), The importance of imperviousness, *Watershed Prot. Tech.*

Slayton, J. L., and E. R. Trovato (1977), *Carbonaceous and Nitrogenous Demand Studies of Potomac Estuary*, Annapolis, MD.

Smith, B. K., J. A. Smith, M. L. Baeck, G. Villarini, and D. B. Wright (2013), Spectrum of storm event hydrologic response in urban watersheds, *Water Resour. Res.*, *49*(5), 2649–2663, doi:10.1002/wrcr.20223.

Smith, E. M., and R. Benner (2005), Photochemical transformation of riverine dissolved organic matter: effects on estuarine bacterial metabolism and nutrient demand, *Aquat. Microb. Ecol.*, *40*, 37–50, doi:10.3354/ame040037.

Smith, R. M., and S. S. Kaushal (2015), Carbon cycle of an urban watershed: exports, sources, and metabolism, *Biogeochemistry*, *126*(1–2), 173–195, doi:10.1007/s10533-015-0151-y.

Srinath, E. G., T. B. S. Prakasam, and R. C. Loehr (1976), Nitrifying Organism Concentration and Activity, *J. Eng.*, 449–463.

Tranvik, L. J. et al. (2009), Lakes and reservoirs as regulators of carbon cycling and climate, *Limnol. Oceanogr.*, doi:10.4319/lo.2009.54.6_part_2.2298.

Tremblay, L., and J. P. Gagné (2009), Organic matter distribution and reactivity in the waters of a large estuarine system, *Mar. Chem.*, doi:10.1016/j.marchem.2009.09.006.

Tufford, D. L., C. L. Samarghitan, H. N. McKellar, D. E. Porter, and J. R. Hussey (2003), Impacts of urbanization on nutrient concentrations in small southeastern coastal streams, *J. Am. Water Resour. Assoc.*, *39*(2), 301–312, doi:10.1111/j.1752-1688.2003.tb04385.x.

U.S. Congress (2002), *Federal water pollution control act*.

U.S. EPA (1995), Technical Guidance Manual for Developing Total Maximum Daily Loads, in *Book II: Streams and Rivers*, p. 254, Washington, DC.

U.S. EPA (2013), *Report on the Performance of Secondary Treatment Technology*.

US EPA (1995), Technical Guidance Manual for Developing Total Maximum Daily Loads, *United States Environ. Prot. Agency, Off. Water*, doi:EPA 823-B-95-007.

US EPA (2016), About NPDES, *Natl. Pollut. Disch. Elimin. Syst.*

Waccamaw Regional Council of Governments (2014), *2014 Murrells Inlet Watershed Plan: A Community-Based Management Plan to Address Fecal Coliform Impariments in Local Shellfish Harvesting Areas*.

Wahl, M. H., H. N. McKellar, and T. M. Williams (1997), Patterns of nutrient loading in forested and urbanized coastal streams, *J. Exp. Mar. Bio. Ecol.*, *213*(1), 111–131, doi:10.1016/S0022-0981(97)00012-9.

Wallace, T. A., G. G. Ganf, and J. D. Brookes (2014), Rapid utilisation of storm water-derived dissolved organic carbon and its fractions in an urban lake, *Mar. Freshw. Res.*, *65*(4), 370–377, doi:10.1071/MF12287.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R. P. Morgan (2005), The urban stream syndrome: current knowledge and the search for a cure, *J. North Am. Benthol. Soc.*, doi:10.1899/04-028.1.

Webster, J. R., E. F. Benfield, T. P. Ehrman, M. A. Schaeffer, J. E. Tank, J. J. Hutchens, and D. J. D'Angelo (1999), What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta, *Freshw. Biol.*, *41*(4), 687–705, doi:10.1046/j.1365-2427.1999.00409.x.

Wiegner, T. N., and S. P. Seitzinger (2004), Seasonal bioavailability of dissolved organic carbon and nitrogen from pristine and polluted freshwater wetlands, *Limnol. Oceanogr.*, *49*(5), 1703–1712, doi:10.4319/lo.2004.49.5.1703.

Wiegner, T. N., S. P. Seitzinger, P. M. Glibert, and D. A. Bronk (2006), Bioavailability of dissolved organic nitrogen and carbon from nine rivers in the eastern United States, *Aquat. Microb. Ecol.*, *43*(3), 277–287, doi:10.3354/ame043277.

Williams, C. J., Y. Yamashita, H. F. Wilson, R. Jaffe, and M. A. Xenopoulos (2010), Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems, *Limnol. Oceanogr.*, doi:10.4319/lo.2010.55.3.1159.

APPENDIX A

A.1 Nitrogenous biochemical oxygen demand (NBOD)

BOD is defined as the amount of DO removed during the respiration of organic matter *and* the conversion of nitrogen compounds, specifically NH_4^+ and DON, to the stable forms of NO_3^- and NO_2^-. The nitrification or nitrogenous BOD (NBOD) interference prevents a direct comparison between BOD rate kinetics with carbon degradation or carbonaceous BOD (CBOD). The Ultimate BOD Test standard method (5210 C.) offers suggestions for nitrification inhibitors such as 2-chloro-6(trichloromethyl) pyridine (TCMP) or allylthiourea (ATU) solution but cautions that such inhibitors should *not* be added to natural samples due to potentially inconsistent results [*Eaton et al.*, 2005]. Instead the standard method recommends monitoring the concentration of nitrite and nitrate species every five days throughout the incubation in order to compute NBOD [*Eaton et al.*, 2005]. This recommended method poses several issues: bottles must be opened every 5 d disrupting the high resolution time course DO measurements made possible by the chemiluminescent spot system; additional sample volume is required to replace each aliquot removed for nitrogen analyses; and collection and hand-filtering samples every 5 d is time intensive.

To address these shortcomings, only a subset of samples (n=8 without nutrients; two of each catchment classification) were monitored for NBOD by conducting parallel incubations. On days 0, 2, 5, 10, 20, and 28 samples were aliquoted from the parallel

incubation bottles and syringe filtered through a 0.7 μm GF/F immediately following a DO measurement. DON, NH_4^+, NO_3^- and NO_2^- were determined as previously described in the *Methods section*. The concentrations of NO_3^- and NO_2^- were converted to DO concentrations using the conversion factors of 4.57 and 1.14, respectively [*Eaton et al.*, 2005]. NBOD at each time point was determined by the same simple subtraction as described previously in Equation 1 and was normalized to total BOD.

During the first five days, NBOD generally accounted for as little as 0 to 3% of total BOD (Figure A.1), which falls within the analytical error of precision associated with the BOD5 incubations. The %NBOD in a sample collected at site 3, a forested stream, with a four-fold higher initial NH_4^+ concentration, did exceed this range (%NBOD= 7 to 54%). Beyond 5 d the contribution of NBOD only increased to as high as 12.9 to 38.3% on day 20 or 28 (Figure A.1), apart from the high ammonium outlier sample. This increase in NBOD over time is consistent with the slow growth rate and often small initial population of one of the most common nitrifying bacteria *Nitrosomonas spp* that carry out the first reaction involved in nitrification (NH_4^+ to NO_2^-) [*Srinath et al.*, 1976; *Slayton and Trovato*, 1977]. Figure A.2 shows the change in average %NBOD over time with the outlier removed. This data presumably follows first order kinetics, but the small number of data collected during the final days of the incubation hinder the fit.

Adjustments for NBOD are not needed to obtain the 5 d CBOD, but NBOD should be considered when the aim is to measure ultimate CBOD. Due to the small sample size of NBOD measurements, no adjustments were made in this study. All UBOD values whether measured over 28 d or calculated account for both CBOD and NBOD.

Regardless both fractions contribute to the removal of oxygen in aquatic systems. It is noted that the degradation kinetic curves of only 15 samples exhibited the traditional two-pool step in their kinetic curves. First order kinetics could not be used to determine UBOD in two of these samples (see Results section), another three (circled in Figure 3.5; Table 3.1) had k-values < 0.02, and the other ten contained > 0.24 mg L^{-1} (~14 μM) NH_4^+.

A.2 BOD nutrient additions

BOD measurements in 2018 were subject to a nutrient treatment as described by the standard methods in order to prevent nutrient limitation over the course of the incubation [*Eaton et al.*, 2005]. Each sample contained an additional 59.24 mg L^{-1} PO_4^{3-}, 1.14 mg L^{-1} NH_4^+, 0.41 mg L^{-1} Mg^{2+}, 9.94 mg L^{-1} Ca^{2+}, and 0.09 mg L^{-1} Fe^{3+}. Nutrient additions could enhance microbial degradation of OM (CBOD) by eliminating nutrient stress that may prevent anabolism of microbial cell biomass [*del Giorgio and Cole*, 1998]. The nutrient addition of NH_4^+, however, has the potential to also promote nitrification (NBOD) [*Slayton and Trovato*, 1977; *Eaton et al.*, 2005]. Therefore, the addition of nutrients during incubations in 2018 could produce larger BOD values than the natural concentrations. Although incubations conducted without nutrient additions, such as those conducted in 2019, may under predict natural BOD concentrations, by assuming that a parcel of water is never resupplied nutrients. The true value likely lies somewhere in between; this is simply a caveat of lab based incubation studies.

To evaluate the difference between BOD incubations with and without nutrient additions, parallel incubations were conducted during 2018 and 2019 (n=18). BOD5 values proved similar regardless of nutrient treatment (Figure A.2; $p > 0.1$). Conversely,

measured UBOD concentrations in samples with nutrient additions were significantly larger than the measured UBOD concentrations of samples without nutrients additions (Figure A.2; $p \leq 0.001$). Bottles with added nutrients almost consistently had BOD values that were 2 mg L^{-1} higher than those without nutrients. This lag time associated with the change in BOD concentrations suggests NBOD is responsible for the difference; NBOD has been termed second stage BOD due to the slow growth rate of nitrifying bacteria [*Slayton and Trovato*, 1977]. The 2 mg L^{-1} difference in UBOD values can also be accounted for under the assumption that all of the additional 1.14 mg L^{-1} NH_4^+ was converted to NO_3^- over the 28 d incubation. Further, monitoring the contribution of NBOD to total BOD in samples treated with nutrients (n=8) as described in Appendix A.1, shows that NBOD is a negligible contribution on days 0 through 5, but may account for as much as 20-90% of the total BOD on days 10, 20, and 28. This is a much higher contribution than observed in samples without nutrients (see Appendix A.1). Finally, all measured UBOD curves in this nutrient rich samples mimicked the standard two pool curve described by many [*Slayton and Trovato*, 1977; *Delzer and Mckenzie*, 2003; *Eaton et al.*, 2005] as an indication of nitrification. Together these findings point to nitrification as the cause of higher UBOD values, though slightly enhanced microbial respiration cannot be fully ruled out.

Since the goal of this study was to understand natural stormwater BOD concentrations and relate these concentrations to carbon degradation, the UBOD values and degradation kinetics measured in 2018 were not reported. It introduced uncertainty by increasing the concentration of a second pool, the nitrogenous fraction, that contributes to BOD concentrations. More thorough investigation into the role of nutrients

in enhancing BOD is necessary to support a better standard UBOD method and understand the effects of inorganic nutrient pollution on BOD concentrations. From this limited comparison and other findings discussed in the main text, irrespective of the biochemical processes, high inorganic nutrient concentrations, especially NH_4^+, tend to increase BOD concentrations and should be a focus of stormwater management.

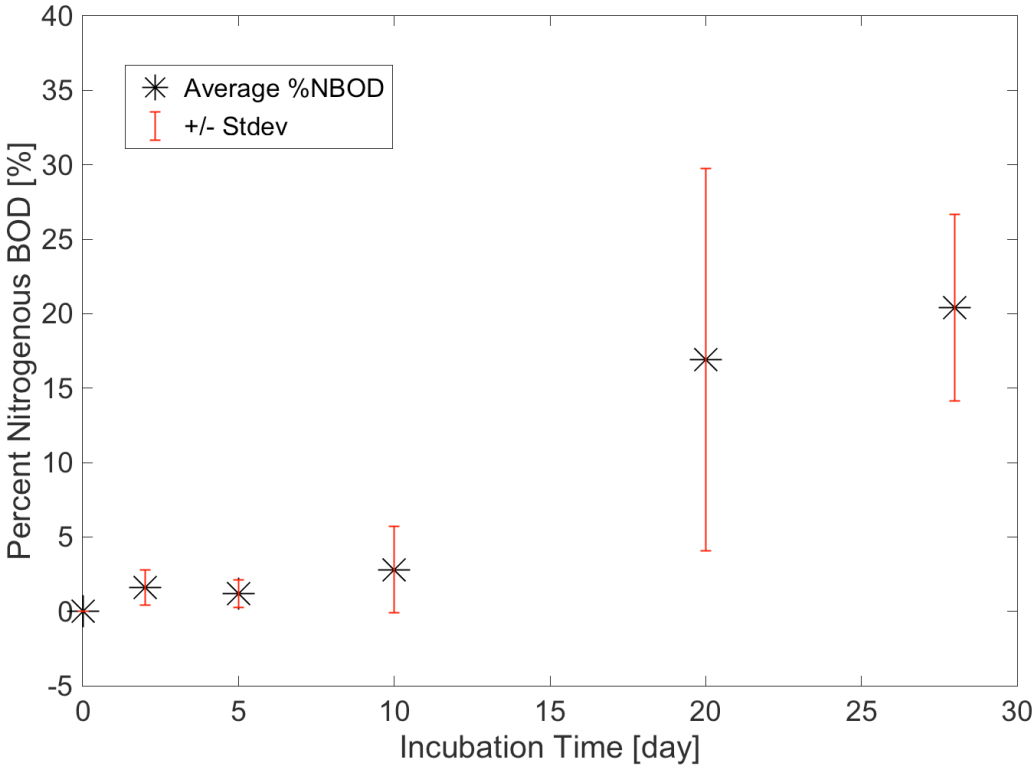

Figure A.1 The average amount of BOD attributed to oxygen removal from nitrification (NBOD) expressed as the percent of the total BOD measured (n = 8 without nutrients) over the course of the 28 d BOD incubation. NBOD was monitored by tracking the changes in nitrate and nitrate species. Red lines indicate the error (± 1 standard deviation) associated with the average percent NBOD.

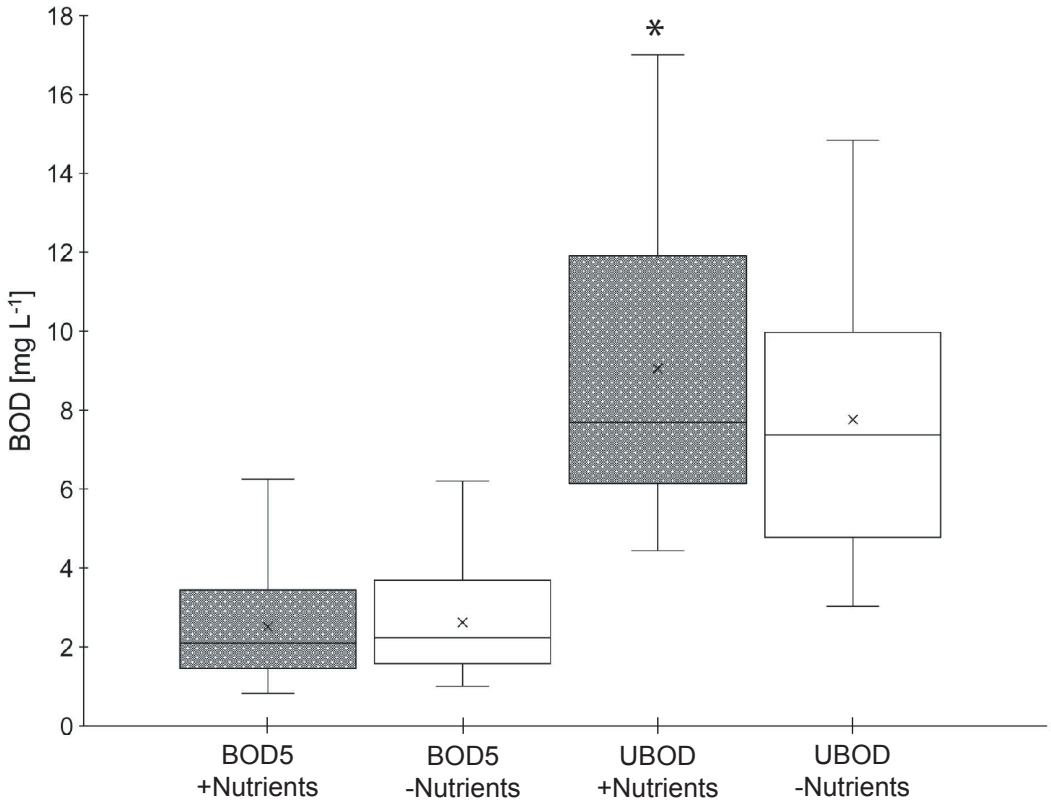

Figure A.2 The boxes represent the range of BOD5 and BOD28 values of two sample treatments. The unfilled boxes are representative of samples with initial *in situ* nutrient concentrations while the shaded boxes are representative of samples treated with additional nutrients. There is not a statistically significant difference between BOD5 values for the two treatments ($p = 0.442$), however the difference in BOD values becomes statistically significant as the incubation time increases to 28 days ($p \leq 0.001$; marked with asterisks).